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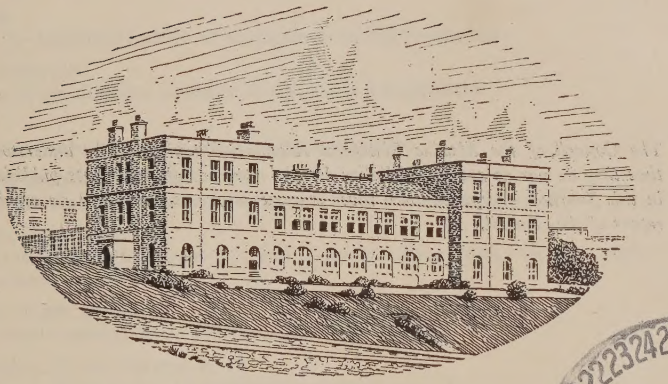
JOURNAL OF THE
MARINE BIOLOGICAL ASSOCIATION
OF THE UNITED KINGDOM

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)
and published by the Cambridge University Press
Cambridge, and Bentley House, London

Agents for Canada and India: Macmillan

JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

1684⁶⁰



THE PLYMOUTH LABORATORY

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VOLUME XXXII

1953

CAMBRIDGE
AT THE UNIVERSITY PRESS
1953

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CONTENTS OF VOLUME XXXII

NUMBER 1, pp. 1–271, JUNE 1953
NUMBER 2, pp. 273–548, OCTOBER 1953

PAGE

Report of the Council:
1952–53 527

Income and Expenditure Account and Balance Sheet:
1952–53 546

ALEXANDROWICZ, J. S. and CARLISLE, D. B.
Some experiments on the function of the pericardial organs in Crustacea . . 175

BAINBRIDGE, R.
Studies on the interrelationships of zooplankton and phytoplankton . . . 385

BALLANTINE, DOROTHY
Comparison of the different methods of estimating nanoplankton 129

BARNES, H.
Size variations in the cyprids of some common barnacles 297

BARNES, H. and BARNES, MARGARET
Biometry of the copepod *Calanus finmarchicus* (Gunn.) in stages V and VI . . 305

BARNES, H. and POWELL, H. T.
The growth of *Balanus balanoides* (L.) and *B. crenatus* Brug. under varying conditions of submersion 107

BRULL, L. and NIZET, E.
Blood and urine constituents of *Lophius piscatorius* L. 321

BRULL, L., NIZET, E. and VERNEY, E. B.
Blood perfusion of the kidney of *Lophius piscatorius* L. 329

CARLISLE, D. B.
Moulting hormones in *Leander* (Crustacea Decapoda) 289
See Alexandrowicz, J. S. and Carlisle, D. B. 175

CHIPPERFIELD, P. N. J.
Observations on the breeding and settlement of *Mytilus edulis* (L.) in British waters 449

DAVENPORT, DEMOREST
Studies in the physiology of commensalism. III. The polynoid genera *Acholoë*, *Gattyana* and *Lepidasthenia* 161
Studies in the physiology of commensalism. IV. The polynoid genera *Polynoë*, *Lepidasthenia* and *Harmothoë* 273

FORSTER, G. R.
A new dredge for collecting burrowing animals 193
Peritrophic membranes in the Caridea (Crustacea Decapoda) 315
The spawning behaviour of plaice 319

FOUNTAIN, H. C.
The examination of the original slides of marine Acari of Hodge, 1863 . . . 357

FRETTER, VERA
Experiments with radioactive strontium (^{90}Sr) on certain molluscs and polychaetes 367

HOLME, N. A.
The biomass of the bottom fauna in the English Channel off Plymouth . . 1

PAGE

KENNEDY, GILBERT Y.
Chlorocruoroporphyrin: a simple method of preparation 365

KENNEDY, G. Y. and VEVERS, H. G.
The biology of *Asterias rubens* L. V. A porphyrin pigment in the integument . 235

KNIGHT-JONES, E. W.
Decreased discrimination during setting after prolonged planktonic life in larvae of *Spirorbis borealis* (Serpulidae) 337

NEWELL, B. S.
Cellulolytic activity in the lamellibranch crystalline style 491

NICOL, J. A. C.
Luminescence in polynoid worms 65

NIZET, E.
See BRULL, L. and NIZET, E. 321
See BRULL, L., NIZET, E. and VERNEY, E. B. 329

OWEN, G.
On the biology of *Glossus humanus* (L.) (*Isocardia cor* Lam.) 51

PARKE, MARY
A preliminary check-list of British marine Algae 497
Index to Genera 555

POWELL, H. T.
See BARNES, H. and POWELL, H. T. 107

REES, C. B.
The larvae of the Spatangidae 477

SHELBOURNE, J. E.
The feeding habits of plaice post-larvae in the Southern Bight 149

SUTTON, MURIEL F.
The regeneration of the siphons of *Ciona intestinalis* L. 249

VERNEY, E. B.
See BRULL, L., NIZET, E. and VERNEY, E. B. 329

VEVERS, H. G.
See KENNEDY, G. Y. and VEVERS, H. G. 235

WELLS, G. P.
Defaecation in relation to the spontaneous activity cycles of *Arenicola marina* L. . 51

WICKSTEAD, J.
A new apparatus for the collection of bottom plankton 347

WILSON, DOUGLAS P.
Notes from the Plymouth Aquarium. II 199
The settlement of *Ophelia bicornis* Savigny larvae. The 1952 experiments . . 209

ABSTRACTS OF MEMOIRS RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

An experimental study of the scattering of light by natural waters. By W. R. G. Atkins and H. H. Poole 269

D-Amino-acid oxidase in the molluscan liver. By H. Blaschko and Joyce Hawkins . 270

Observations on amine oxidase in cephalopods. By H. Blaschko and Joyce Hawkins 270

PAGE

The mechanical analysis of the responses from the end-organs of the horizontal semicircular canal in the isolated elasmobranch labyrinth. By J. J. Groen, O. Lowenstein and A. J. H. Vendrick 270

Muscle activity and drug action in the body-wall of the sabellid worm *Branchiomma vesiculosum* (Montagu). By J. A. Colin Nicol 271

Receptor elements in the thoracic muscles of *Homarus vulgaris* and *Palinurus vulgaris*. By J. S. Alexandrowicz 521

Notes on the nervous system in the stomatopoda. II. The system of dorsal trunks. III. Small nerve cells in motor nerves. By J. S. Alexandrowicz 521

Resistenz der Meeresalgen gegen sichtbares Licht und gegen kurswellige UV-Strahlen. By R. Biebl 522

Lichttransmissionsänderungen an Meeresalgen, im besonderen an *Porphyra umbilicalis* f. *laciniata*. By R. Biebl 522

Some structural proteins of *Mytilus edulis*. By C. H. Brown 523

Presenza di spicole in *Diplosoma listerianum* (Milne Edwards). Contributo alla sistematica degli Ascidiacea, Didemnidae. (The presence of spicules in *Diplosoma listerianum* [Milne Edwards]. A contribution to the systematics of the Ascidiacea, Didemnidae.) By D. B. Carlisle 523

Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). II. Experimental evidence for a growth- and moult-accelerating factor obtainable from eyestalks. By D. B. Carlisle and P. F. R. Dohrn 524

Plankton of the Benguela Current. By T. J. Hart 524

Contraction and relaxation in the adductor muscles of *Mytilus edulis*. By J. Lowy . 525

CORRIGENDA

Vol. xxxi

Page 501. Fifth and fourth lines from bottom. *For* g. *read* Kg.
502. Line 4. *For* g. *read* Kg.
507. Sixth and seventh lines of Summary. *For* wet weight of phytoplankton *read* chlorophyll.

Vol. xxxii

Page 15. Table IV. For *Nassa* read *Nassarius.*
42. Table XXIV. For *Glycimeris glycimeris* read *Glycymeris glycymeris.*
65. One line from bottom. The second *squamatus* should be *squamata.*
101. Sixth line from bottom. For *chamosolen read chamasolen.*
403. Line 7. *For* chryso- *read* chloro-.
441. Line 16. *For* Foxon *read* Faxon.
527. Seventh line from bottom. For *americanus* read *americanum.*

Vol. xxxii. British Marine Algae Check-list

Page 497. Line 10. *For* 125 *read* 124.
506. L. column, near bottom. After *scorparia* (L.) Sauv. *add inset* (*Stypocaulon scoparium* Kütz.).
506. R. column, third genus. *For* ILOPHUS *read* DILOPHUS.
511. L. column. Line 30. *After* var. *solmsiana* Fkbg. *add inset* (var. *callithamnioides* Fosl.).
511. L. column. Line 41. For *endophlaea* read *endophloea.*
511. L. column. Line 52. *After* CALLOCOLAX *for* Schmitz, 1895 *read* Schmitz ex Batters, 1895.
512. R. column. *After* CORYNOSPORA J. Agardh...1876 *add* (MONOSPORA Solier).
516. L. column. Line 7. *For* (*Xenococcus shousboei* Thur.) *read* (*Dermocarpa schousboei* Born.).

THE BIOMASS OF THE BOTTOM FAUNA IN THE ENGLISH CHANNEL OFF PLYMOUTH

By N. A. Holme
The Plymouth Laboratory

(Text-figs. 1–7)

CONTENTS

PAGE
Introduction 1
Conditions 2
Methods 8
  Collection of samples 8
    Method of collecting 8
    Validity of the samples 9
    Number of samples required 12
    Variability of samples at a station . . . 14
    Seasonal changes 19
    Sieving 20
  Biomass estimations 22
  Nomenclature 25
Details of Stations and Collections 25
Summary of biomass data 37
Changes in the bottom fauna 39
  Allen's survey 39
  Ford's survey 39
  Mare's survey 43
Discussion 43
Summary 46
References 47

INTRODUCTION

The earliest survey of the bottom fauna in the English Channel off Plymouth is that of Allen (1899), who made a large number of dredge hauls in the neighbourhood of the Eddystone. His sampling was necessarily qualitative, and not until a quarter of a century later were any quantitative studies undertaken (Ford, 1923). Following the work of C. G. J. Petersen and others in Denmark, Ford made a survey of the communities near Plymouth, which he found to be *Venus* communities of two types, the occurrence of each type depending upon the grade of deposit.

Many workers have criticized the use of the Petersen grab as a quantitative instrument in deposits other than soft mud. It clearly does not dig deeply enough to sample all the fauna, but it has given valuable results for comparative purposes.

Further quantitative work at Plymouth has been undertaken for more limited areas by Steven (1930) and by Smith (1932), using the Petersen grab and conical dredge respectively. Mare (1942) made a detailed study of the inhabitants of a muddy ground a few miles offshore, with particular reference to the micro-organisms. She gives the only available data on the quantity of living tissue per unit area of the sea-bed at Plymouth.

Elsewhere in the British Isles, Davis (1923, 1925) and Stephen (1923) have studied benthic communities in the North Sea, and more recently Jones (1951), in an account of the communities off the Isle of Man, has given figures for the quantity of living tissue per unit area.

It is well known that in the early nineteen-thirties the productivity of the sea in the western part of the English Channel started to decline. This change, associated with the replacement of a water-mass characterized by the planktonic chaetognath *Sagitta elegans* with one characterized by *S. setosa*, may be the cause of a subsequent decline in the numbers of young fish caught in a standard net, and probably also in a decrease in the populations of other organisms. Unfortunately, there are usually insufficient data to record changes in population densities, and a need was felt for further quantitative data by means of which any future changes in productivity could be followed.

The quantity of life to be found in deposits of sands, gravels or muds on the sea-bed can be more readily assessed than most other forms of life in the sea, and it was felt that a survey of the bottom fauna, using a bottom sampler similar to that previously described (Holme, 1949) would provide a basis for following such changes.

I am indebted to Captain C. A. Hoodless, and the crew of R.V. *Sabella* for their skill in operation of the gear at sea. I would like to thank Dr M. N. Hill for loan of his free-fall core sampler, and Prof. W. B. R. King, F.R.S., for identifying the rock samples obtained with this instrument. Mr F. A. J. Armstrong has given much assistance in suggesting methods for dry-weight determinations, and in other ways. The following have kindly identified or checked certain groups: Mr G. M. Spooner (Amphipoda), Miss P. Kott (Tunicata), Dr H. G. Vevers (Echinodermata), and Dr C. Burdon-Jones (Enteropneusta).

CONDITIONS

The area sampled is situated in the English Channel within 16 miles of Plymouth, and lies between 50° 6′ N. and 50° 20′ N., and between 4° 5′ W. and 4° 16′ W. The coastline between Lizard Point in Cornwall and Bolt Head in Devonshire sweeps northward to form a large open bay, thus providing slight protection from Atlantic swell, particularly in the western part of the bay, around Falmouth. Off the Eddystone, however, protection is negligible, but tidal streams in the bay are somewhat weaker than in the main part of the Channel to the southward.

Depth. A mile or two off the coast near Plymouth the bottom shelves away steeply from 20 to 40 m., and then slopes down very gently so that in mid-channel, 60 miles to the south, the depth is only 100 m. Except for three stations near shore, the area covered in this survey is on this gently sloping bottom, in depths of 40–70 m. Twenty stations were worked in the survey; they were placed at 2-mile intervals along three lines running southward from the shore (Fig. 1).

Tidal streams. Tidal streams near shore are variable in direction and strength, but out by the Eddystone they set east and west, reaching a velocity of over 1 knot (1829 m./hr.) at spring tides.

Wave action. It is difficult to assess the effects of wave action on the burrowing fauna. Atlantic swell and waves generated in the Channel must cause considerable disturbance in shallow water—in Whitsand Bay, for example, where large lamellibranchs such as *Ensis* are often washed in alive during storms. In deeper water the effects of wave action must be much reduced. Allen (1899) considered that the abundance of hydroids and the polyzoan *Cellaria*, which root themselves in the sand, was evidence that the grounds in the neighbourhood of the Eddystone were not subjected to violent disturbances at any time. In the immediate vicinity of the Eddystone reef, however, there is evidence of considerable wave action and tidal scour (Allen, 1899, p. 376; Smith, 1932, p. 253).

Deposits. The texture of the deposits is very variable and at certain stations inside the Eddystone a small change in position due to the ship drifting between one haul of the bottom-sampler and the next often results in a marked change in the nature of the sediment brought up (e.g. at stations A3 and A4). Samples of deposit, as brought up in the bottom-sampler, have been mechanically graded. The Wentworth grade scale has been adopted, and the soils have been graded through gauze sieves of the following apertures: 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{5}$ and $\frac{1}{8}$ mm. A separation of particles over and under $\frac{1}{32}$ mm. was made by sedimentation and repeated decantations. (The method is not described here in detail: further work involving mechanical analysis of soils in relation to their lamellibranch faunas is in progress, and a detailed account will be published with this work.)

The results of mechanical analysis are given in Table I, and cumulative curves for some stations are shown in Fig. 2.

Close inshore, in Whitsand Bay, a fairly clean sand is found; farther offshore this gives way to a patchwork of grounds extending out as far as the Eddystone. Off Whitsand Bay is the 'Rame mud' which is in fact largely composed of fine sand (60% between $\frac{1}{8}$ and $\frac{1}{32}$ mm. grade at A2). Farther out the deposits range from coarse muddy sand to fine gravel mixtures: these two types are found both at A3 and A4. Where tidal scour is strong, there may occur patches of gravel, often largely composed of shell-remains and so forming a 'shell-gravel'. A typical shell-gravel occurs close to the Eddystone

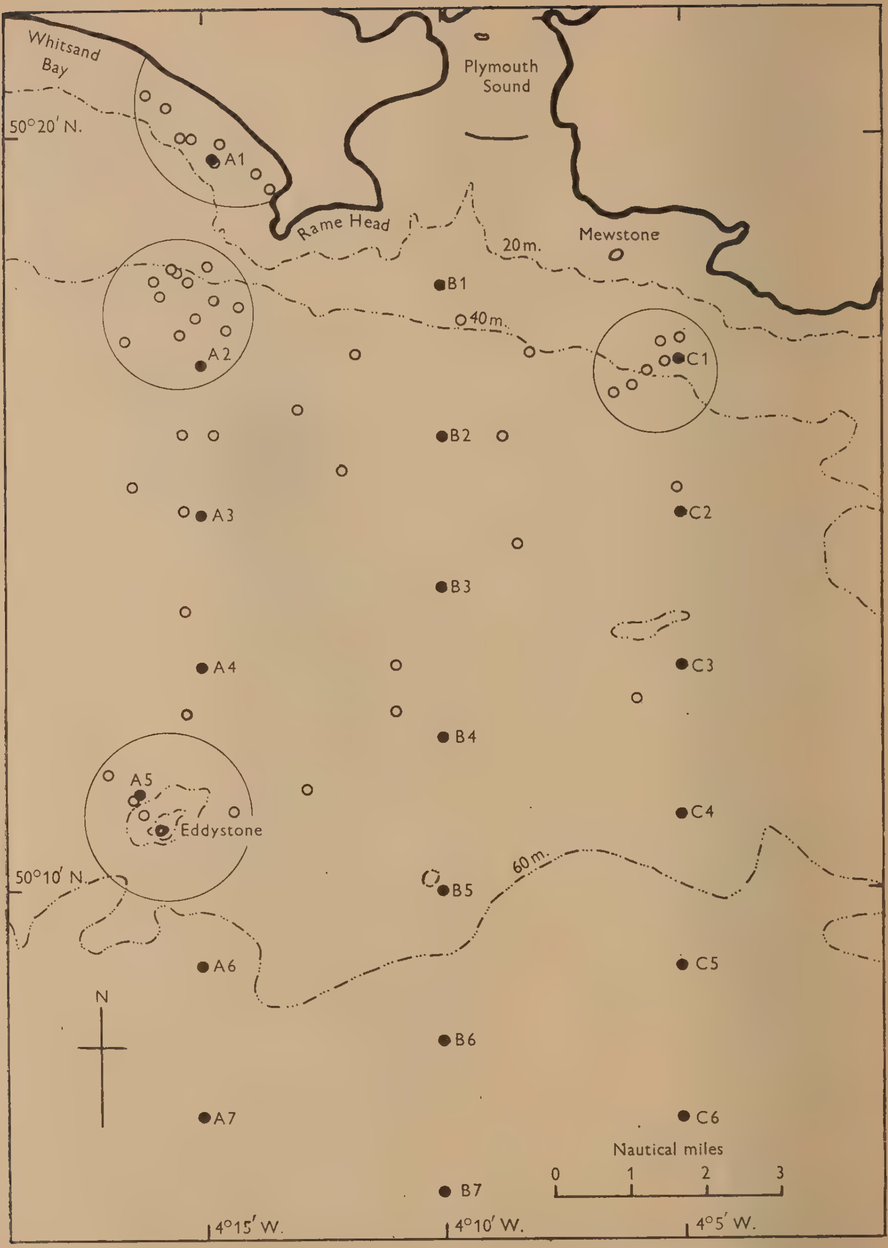
Whitsand Bay
50°20′ N.
Plymouth Sound
Rame Head
20m.
Mewstone
A1
A2
B1
40m.
C1
B2
A3
C2
B3
A4
C3
B4
A5
C4
Eddystone
50°10′ N.
60 m.
B5
A6
C5
N
B6
A7
C6
Nautical miles
0
1
2
3
B7
4°15′ W.
4°10′ W.
4°5′ W.

Fig. 1. Chart of the English Channel off Plymouth. Depth contours in metres. ●, 1950 stations; ○, Ford's stations; the four areas where samples have been grouped together (see p. 40) are enclosed in circles.

(A5), and there are other patches off Plymouth Sound (B1) and off the Mewstone (C1). In such places there are probably outcrops of bare rock in pockets of which the shell gravel rests. To the south and east of the Eddystone lies a fairly clean fine sand, its composition varying little from station to station (A6, 7, B4, 5, 6, 7, C4, 5, 6.)

Nowhere was the soil below the surface found to be black from the presence of ferrous sulphide, and the soils therefore may be assumed to be well oxygenated. I have found this the usual condition of soils in the English Channel. The only soil so far found to be black below the surface was in Torbay (p. 11), where tidal streams are weak and stagnant conditions resulting from deposition of organic matter may arise from time to time.

TABLE I. SOIL GRADES, EXPRESSED AS PERCENTAGES BY WEIGHT

(Figures in brackets after station numbers indicate the serial number of the haul.)

	Grade (mm.)							
Station	>2	2–1·0	1·0–0·5	0·5–0·25	0·25–0·20	0·20–0·125	0·125–0·0313	<0·0313
A1	0·07	0·37	1·11	6·09	8·62	63·74	19·76	0·22
A2	0·13	1·29	1·87	4·06	1·67	11·14	60·27	19·58
A3 (1–2)	0·71	1·20	10·35	26·54	5·49	35·95	18·24	1·52
A3 (3–4)	28·90	14·18	9·81	9·00	1·62	12·31	17·15	7·03
A4 (1)	1·20	2·99	9·56	30·57	7·77	25·96	19·04	2·90
A4 (2)	27·73	10·86	7·73	13·73	3·29	13·29	17·69	5·69
A5	49·16	38·04	9·36	2·03	0·18	0·35	0·52	0·35
A6	0	0·15	2·37	14·59	7·70	61·63	12·30	1·26
A7	0·07	0·20	1·92	17·98	6·37	63·04	9·36	1·06
B1	48·03	32·05	16·51	1·48	0·09	0·35	0·87	0·61
B2	27·05	8·08	7·03	9·22	2·07	11·48	26·46	8·60
B3	0·32	1·60	4·87	6·85	1·79	44·01	36·19	4·36
B4	0·45	0·53	3·04	10·22	3·82	55·87	23·48	2·59
B5	0·07	0·75	3·40	7·34	10·81	59·28	16·93	1·43
B5*	0·44	0·89	3·34	9·39	12·54	60·01	12·59	0·79
B6	0·06	1·07	3·74	11·87	6·53	62·73	13·00	1·01
B7	0	0·16	1·81	14·90	6·74	67·01	8·11	1·26
C1 (3–4)	14·45	41·80	37·75	4·22	0·25	0·29	0·63	0·63
C2 (3)	6·93	8·45	7·19	14·32	3·10	21·56	32·58	5·88
C3 (2)	21·32	16·73	4·18	2·43	0·47	6·95	36·03	11·88
C4	0	0·15	0·66	2·93	4·75	69·63	19·76	2·12
C4†	0·07	0·82	0·67	1·80	2·10	55·17	38·38	0·97
C5	0	0·05	0·31	6·55	11·27	67·35	13·26	1·21
C6	0	0·15	0·95	11·66	6·82	69·94	9·53	0·95

* 5 December 1950 (hauls 37–38). † 7 November 1950.

The low percentage of silt (<0·0313 mm.) in the deposits outside the Eddystone suggests that there is little or no *net* deposition in the area. Thus benthic filter- and deposit-feeding organisms cannot be dependent on a continual rain of dead organisms, faecal pellets or other organic detritus from the overlying water, except possibly during calm weather and at neap tides when there is a minimum of disturbance near the bottom. Normally such detritus must stay in suspension to be fed on by filter-feeding planktonic and benthic organisms or by bacteria. These conclusions are supported by the work of Armstrong & Harvey (1950), who found that water samples taken very close to the bottom had a phosphorus content only a little higher than that of the overlying water. From this it was inferred that there was no material deposit of detritus rich in phosphorus.

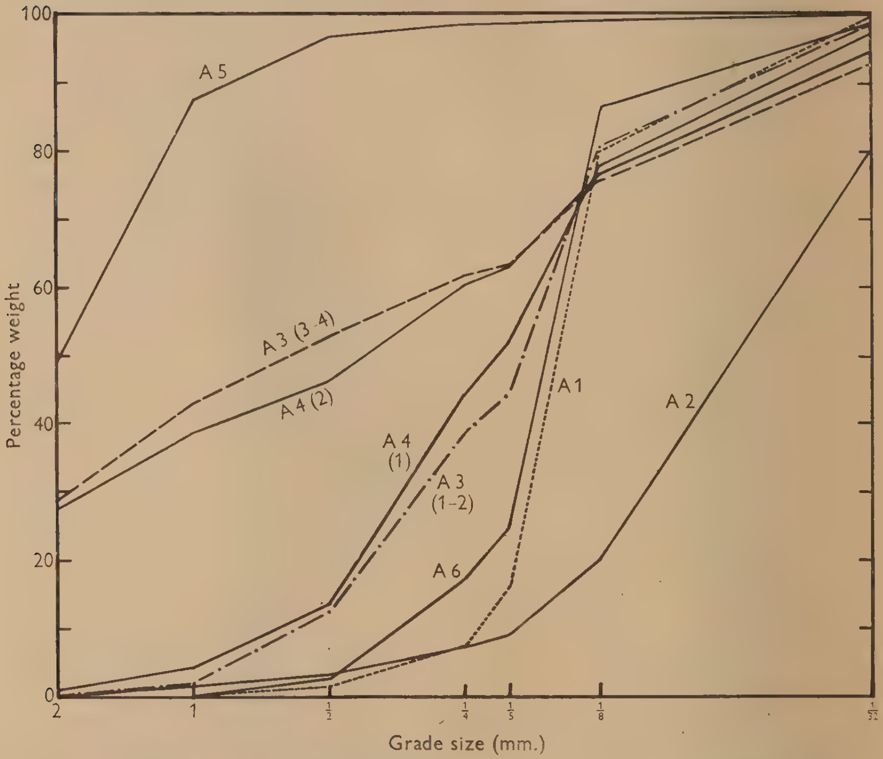

Fig. 2. Cumulative curves of the soil grades of stations on the 'A' line. Note the variation in soil grade in different hauls at A3 and A4. The soil at A7, which is not included, is similar to that at A6.

Depth of sediment. Until lately, nothing was known of the depth of sediment overlying the solid rock forming the floor of the English Channel. Recent work by King (1950) and by M. N. Hill, using a modified Stetson free-fall corer, has shown that there is only a thin veneer of sediment in many places in the western half of the Channel.

Core-samples have been taken at seventeen of the twenty stations worked in the benthos survey, and rock has been found at eleven of these (Table II). When the coring tube strikes rock it often penetrates a short distance, the rock breaking off and forming a plug in the jaws of the tube. In this way a core of the overlying sediment may be obtained. If the coring tube fails to enter rock a core of sand is often retained by an arrangement of flap-valves near the mouth of the tube. The depth of the sediment has been calculated from the volume of the sample. Where no rock was taken this represents the minimum depth of sediment. Possibly some of the depths recorded at stations where no rock was taken represent the total depth of sediment, the corer not having sufficient momentum after passing through 30 cm. or more of sand to penetrate the rock.

TABLE II. DEPTH OF SEDIMENT AND GEOLOGICAL NATURE OF THE UNDERLYING ROCK

	Depth of sediment (cm.)	Rock
A1 a*	*c.* 1	Devonian slates
b*	18·0	Devonian slates
A2	10·4	Red rubble, probably N.R.S.
A3	>71·5	..
A4	>33·8	..
A6	21·8	Red rubble breccia with limestone fragments. Almost certainly N.R.S.
A7	32·2	Fine red rather marly N.R.S.
B3	19·4	Breccia, mainly small slate fragments. Almost certainly N.R.S.
B3/4†	> *c.* 50	..
B4	36·0	Breccia of quartzite fragments. N.R.S.
B5	33·3	Firm fine N.R.S.
B6	>25·0	..
B7‡	21·7	Fairly coarse N.R.S.
C1	19·4	Fine, typical, N.R.S.
C2	31·4	Very fine loamy N.R.S.
C3	0	Mica schist
C4	>33·3	..
C5	>38·9	..
C6	>27·8	..

* Sample *a* from 100 yards west of the wreck in Whitsand Bay, sample *b* from 100 yards north-west of the wreck.

† Mid-way between B3 and B4.

‡ Three previous drops had failed to take a rock sample.

N.R.S., New Red Sandstone.

Estimated depths of sediment are shown in Table II. At C3 rock is exposed at the surface, and at A1 the sediment was only 1 cm. deep in one haul. At the other stations where rock was taken the minimum depth was 10·4 cm., and the mean 25·0 cm. Although most of the burrowing fauna is believed to be present in the top 10 or 20 cm. of soil, it is evident that the shallowness of the soil may often restrict the occurrence or growth of deeper burrowing species, some of which are thought to penetrate at least 30 cm. into the sediment.

METHODS

Collection of samples

Method of collecting

Samples were taken with a modified form of 'scoop-sampler', as already described (Holme, 1949), but having two counter-rotating scoops instead of the single one (Fig. 3). 54 kg. of weights were added to the frame, bringing the total weight to about 115 kg. It was hoped that the use of two scoops would eliminate the lateral movement of the frame during digging, and so produce a larger sample. Samples taken with the new instrument were, however, not larger than with the previous instrument, but twice the area is sampled at each haul. In addition, the faunas taken by the two scoops can be compared, to give data on the degree of patchiness of the bottom-fauna.

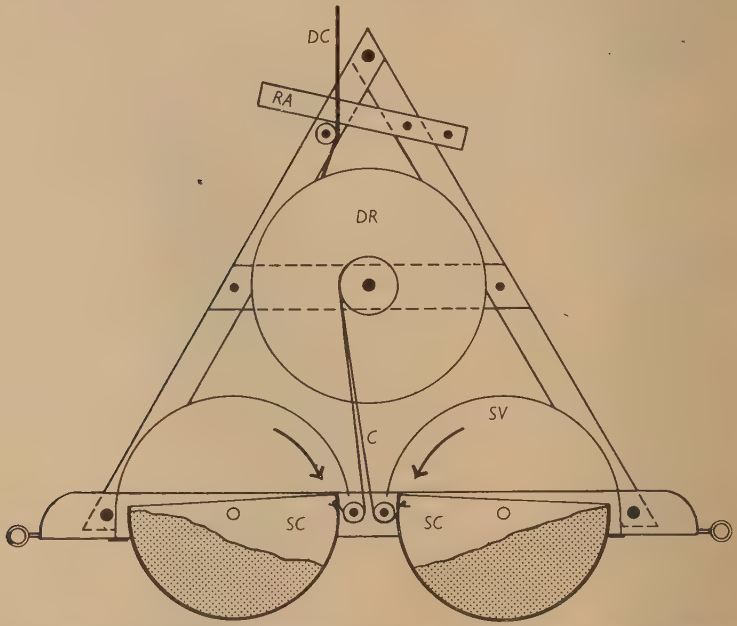

Fig. 3. Diagram of the double-scoop sampler (cf. Holme, 1949, Figs. 1 and 2). The scoops are in the closed position. *C*, small cables which pull the scoops round; *DC*, the main cable which has unwound from the drum and by which the sampler is hoisted; *DR*, drum; *RA*, release arm; *SC*, scoops; *SV*, scoop-covers. The soil sample is stippled.

Each scoop samples a rectangular area which in vertical section is nearly semicircular, like half a cheese. If the scoop is digging to its fullest extent an area 36 × 15·3 cm. is sampled, which is equal to 0·055 m.^{2}, in practice the scoops do not usually dig to the maximum depth of 15 cm., and a correspondingly smaller surface area is covered. In addition, the area sampled decreases with depth in the sediment. For practical purposes each scoop is assumed to sample 0·05 m.^{2}.

No doubt the scoops would dig more deeply in the firmer sediments if the frame were more heavily weighted, but the weight of the apparatus is limited by the size of the ship and the facilities available for handling it on board.

Five hauls were made with the sampler at each station, covering a total area of 0·5 m.^{2}. The ship was allowed to drift while the hauls were being taken, but as sampling was always carried out in fine weather the amount of drift was not great. Once or twice a marker float was anchored at the original position, and the ship was found to drift not more than $\frac{1}{4}$–$\frac{1}{2}$ mile during the 20–30 min. necessary to take the five samples (see p. 14 and p. 29, station B2).

On grounds within a few miles of the shore it is possible to drift over a variety of types of bottom-soil while sampling at a station. On these grounds the soils are sometimes so patchy that a drift of only a few yards may result in a complete change in the nature of the sample brought up (see also Ford, 1923, p. 167). On such grounds the fauna listed for any one station may therefore represent samples from the different 'communities' characterizing the various grades of bottom soils. For the purpose in hand, of assessing the total quantity of life on the sea-bed, this is no great disadvantage, but it would be unwise to attempt to correlate the distribution of species at these stations with the soil grades as shown in Table I. Under such conditions each haul would have to be treated as a separate sample.

On grounds farther offshore, the drift of the ship while on station has no appreciable effect on the fauna brought up (see p. 30).

Validity of the samples

The volume of soil brought up by the sampler is some indication of the depth to which it is digging, but since there is little information on the vertical distribution of species in the soil its efficiency can only be judged against that of other instruments.

It is well known that on soils other than mud the 1/10 m.^{2} Petersen grab does not dig deep enough to sample all the fauna. Johansen (1927) gives an interesting comparison between the performances of the Petersen grab and the Knudsen sampler, which can dig to a depth of 30 cm. in sand. It has already been shown (Holme, 1949) that the 'scoop-sampler' samples sandy grounds more efficiently than does the Petersen grab, and a further comparison is given in Table III.

From observations on intertidal banks it is known that the majority of individuals are to be found in the top 15 cm. or so, and Johansen (1927), working with the Knudsen sampler in shallow water, considers that 'invertebrates are not taken beyond about 12–25 cm. down in the sea floor'.

A brief survey by Molander (1928) of the vertical distribution of the fauna in Gullmar Fjord, using a square coring instrument with an occluding shutter, indicates that the majority of species and individuals are to be found in the top 5 cm. of the sediment, few occurring below 10 cm. The maximum depth of sampling was only 15 cm., however, so some deep-living species may have been missed.

In general it is probably safe to assume that most individuals occur in the top 15 cm., but the few living below this depth are mostly rather large forms and sometimes must contribute largely to the total weight of animal tissue per unit area.

Undoubtedly the 'scoop-sampler', which digs to a maximum of 15 cm., misses deep burrowing forms, such as *Upogebia* and *Callianassa*—which are probably much more numerous than the figures given in this paper suggest. For some species, however, the *effective* sampling depth is greater than 15 cm., as with large lamellibranchs (particularly *Lutraria*) whose siphons are from time to time taken in the scoop. These animals live perhaps 30 cm. down in the sediment and are seldom taken, but from the size of the severed siphon the size of the whole animal can be estimated.

It is uncertain to what extent active surface-living forms can evade the sampler. Small fish, prawns, etc., can probably dart out of the way as the sampler descends, but it seems unlikely that crabs or scallops (*Pecten*, *Chlamys*) can move fast enough to escape. Once the sampler reaches the sea-bed its construction makes it difficult for them to escape.

There is a small loss of soil and possibly of animals as the sampler reaches the surface and the water in the scoop-cover empties out around the scoop; and once on board the water with contained animals may slop on the deck, particularly if there is any swell at the time. These losses, however, are considered to have been small.

While testing a new 'suction'-coring apparatus (Fig. 4), a comparison was made between the number of individuals taken against those taken at the same place with the $\frac{1}{20}$ m.^{2} single-scoop sampler and a $\frac{1}{10}$ m.^{2} Petersen grab. Although the suction-corer is not in general use, mainly because it is rather difficult to handle on board ship, a brief description is included here.

The corer consists of coring-tube (*C*) 36 cm. long and of 16·25 cm. internal diameter, the top of which is in communication with an air chamber (*P*). This assembly is supported by a pipe frame, which rests on the sea-bed and through which the assembly slides into the sediment. It is lowered from the stationary ship, and when it strikes the bottom the tap (*T*) is opened, to give a negative pressure in the coring tube as water rushes in to compress the air in the chamber. It takes about 30 sec. for all the water to enter the chamber, and during this time the coring tube is sinking into the sediment, aided by the reduced pressure inside the tube. The release (*R*) at the top of the chamber has disconnected from the main warp when the cable slackened so that, on hauling up, a lifting force is transmitted to the lifting arms (*LA*), which are nearly horizontal at this stage. As the coring tube is pulled out of the sediment the whole sampler turns over and finally swings under its own weight to bring the open end of the coring tube uppermost. The sample is retained in the coring tube by the sheet of gauze (*G*), but the open end of the tube is not covered. As the sampler comes up, the air in the chamber expands under the reduced pressure. The tap (*T*) has previously closed as the instrument was inverted, and the air is blown off to the exterior through a small valve.

The suction produced was found to increase penetration into the sediment, but penetration would probably have been greatly improved if the coring assembly had been more heavily weighted. The volume of the sample is very little less than would be expected from the measured depth of penetration, there being apparently little loss while the sampler is turning over.

The sampler resembles that of Knudsen (1927), except that suction is produced by hydrostatic pressure instead of by a pump, and that there is a frame supporting the instrument on the bottom.

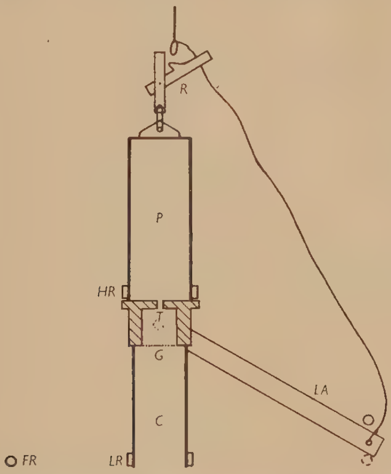

Fig. 4. Diagram of the suction-corer in vertical section. Many details have been omitted. *C*, coring tube; *FR*, pipe frame, which rests on the sea bed, and which is connected to the upper (*HR*) and lower (*LR*) metal rings through which the coring assembly slides. *G*, gauze covering top of coring tube; *LA*, lifting arms, the position of the bolts on which these rotate is dotted. The pipe frame has an upward U-bend to make room for the end of the lifting arms. *P*, pressure chamber; *R*, release; *T*, position of tap mechanism.

The sample taken by this or any other coring tube is more satisfactory in that the cross-sectional area is uniform at all depths in the sample, whereas in the scoop-sampler it decreases with depth.

Details of the comparative hauls are given below:

16. xi. 1949. Brixham Breakwater, light bearing 145° T., 1·25 sea miles (middle of Torbay). Depth: 10·5 m. (from chart).

R.V. *Sabella* anchored. Fine muddy sand, black below top 2–3 cm. 1·2 mm. mesh sieve.

(i) Suction-corer ($\frac{1}{50}$ m.^{2}), fourteen hauls, total area 0·28 m.^{2}. Length of cores (cm.): 20, 14, 13, 29, 20, 15, 7·5, 9, 11·5, 16·5, 21·5, 21·5, 16·5, 18 (the variation in length of the cores is partly due to experimental alteration in the rate and timing of the 'vacuum' release). Mean length: 16·6 cm.

(ii) Petersen grab ($\frac{1}{10}$ m.^{2}), five hauls, total area 0·5 m.^{2}. The mean volume of the sample corresponded to a 'bite' of about 3 cm.

(iii) Single-scoop sampler ($\frac{1}{20}$ m.^{2}), with 50 kg. weights. Total area: 0·25 m.^{2}. Samples about 3 l. each, corresponding to a maximum penetration of 7–10 cm., and a mean penetration of 6 cm.

Table III. Comparison of Faunas taken by the $\frac{1}{50}$ m.^{2} Suction-corer, $\frac{1}{10}$ m.^{2} Petersen Grab, and the $\frac{1}{20}$ m.^{2} Scoop-sampler. Densities per m.^{2}

	Suction-corer	Petersen grab	Scoop-sampler
Anemones	7	10	12
Aphroditidae	14	..	..
Phyllodocidae	3·5	..	..
Nephthys sp.	28·5	24	44
?*Eone* sp.	7	..	..
Owenia tubes	10·5	..	12
Maldanidae	111	10	148
Notomastus sp.	28·5	2	32
Melinna palmata	278·5	62	268
Amphitrite edwardsi	3·5	..	4
Polychaeta indet.	68	6	96
Phascolion strombi	..	2	..
Nemertinea	7	2	..
Amphipoda	10·5	12	24
Crab (adult and megalopa)	7	..	..
Thyasira flexuosa	32	18	32
Mysella bidentata	10·5	4	8
Venus striatula	..	2	8
Abra alba	10·5	12	8
Cultellus pellucidus	..	2	4
Spisula sp.	3·5	8	..
Lutraria sp. (siphons)	7	..	4
Corbula gibba	..	..	4
Lamellibranchia indet.	..	..	4
Turritella communis	28·5	26	40
?*Eulima glabra*	3·5	..	4
Cylichna sp.	..	..	4
Philine sp.	3·5	2	..
Gastropoda indet.	3·5	..	..
Amphiura filiformis	193	26	216
Synapta	3·5	..	..
Total	883·5	230	976

A comparison of the faunas taken is given in Table III. The number of individuals taken by the suction-corer, which penetrated to about 17 cm., is of the same order as that taken by the scoop-sampler, which had dug half as deeply. This indicates that the majority of species were aggregated in the top 7–10 cm.; *Lutraria*, however, of which only siphons were taken, was out of reach of both samplers. The Petersen grab, which was only digging to about 3 cm. depth, was clearly not sampling the fauna adequately.

Number of samples required

Three series of observations have been made to find the number of hauls necessary for a qualitative evaluation of the fauna. Clearly the number of hauls required will vary with the objects of any investigation and local conditions. At each station the animals taken in each haul were kept separately and identified. At two stations the ship was anchored while the hauls were made; but at B5 the ship was drifting over a ground of fairly even faunistic composition (see p. 19). A series of 100 samples taken by Petersen and Boysen Jensen is included for comparison. These were taken with the $\frac{1}{10}$ m.^{2} Petersen grab at various points in Thisted Bredning, the fauna being similar all over the Broad.

(i) Bigbury Bay. 20. iv. 49. Borough Island, bearing 023° T., 1·0 sea miles. Depth: 25·6 m. (from chart). Muddy sand.

R.V. *Sabella* anchored. Fifteen hauls with the single-scoop sampler ($\frac{1}{20}$ m.^{2}) 1·2 mm. sieve.

(ii) Whitsand Bay. 6. v. 49. Rame Head, bearing 144° T., 1·83 sea miles. Depth: *c.* 9 m. (from chart). Sand with shale fragments. R.V. *Sabella* anchored. Fourteen hauls with the single-scoop sampler, then further anchor chain let out so that ship drifted back a little and a further six hauls made. 1·2 mm. sieve.

(iii) Station B5. 5. xii. 50 (see p. 14).

R.V. *Sabella* drifting. Twenty hauls made with the double-scoop sampler ($2 \times \frac{1}{20}$ m.^{2}). Each scoop-full is considered separately.

(iv) Thisted Bredning. See Petersen & Boysen Jensen (1911), Table I. $\frac{1}{10}$ m.^{2} grab.

The species found in each haul are shown in Tables IV–VI. Not all species have been identified: under 'unidentified Polychaeta' are usually lumped the less easily identifiable worms which are in any case often fragmented after collection, sieving and preserving. The total number of species recorded is therefore a minimum figure.

During identification there is a possibility of bias arising from the order in which the individual samples are examined. Where not every species is identified there is the choice of identifying a species or lumping it with the unidentified forms. Thus an unknown worm in the first haul examined might be identified down to its species, but if the first individual had been found in the last haul it might not have been identified. It is therefore important that the order of the samples be randomized before plotting the total number of species against the area sampled. The records from Whitsand Bay and Bigbury Bay were randomized after identification but before plotting the curves, but the samples from station B5 were examined in a random order.

The cumulative curves (Fig. 5) record the total number of species taken as the area sampled is increased. The curves rise steeply at first; and clearly a sample of less than $\frac{1}{2}$ m.^{2}, the area adopted in the survey, does not give an adequate qualitative sample.

Williams (1950) has analysed the occurrence of species of plants in quadrats. He has shown that if the number of species taken is plotted against the log of area (or number of quadrats), a straight-line relationship is approached. Fig. 6 shows the results of plotting against log area for the sets of samples under discussion. Each gives a straight-line relationship. From the slope of the lines it is possible to predict the number of species that will be taken in increasingly larger samples. Thus doubling the area sampled increases the number of species by the following amounts: St B5, 12; Whitsand Bay, 7; Bigbury Bay, 4½; Thisted Bredning, 2½.

It is doubtful if any useful purpose would be served by analysing the occurrence of so-called 'characteristic' species in these samples since these may be by no means common on the grounds. A species may be characteristic of a community without necessarily being at all abundant, and while no doubt the ideal characteristic species are both restricted to a particular set of conditions and very common where they occur at all, it may be necessary to label a community by species of comparatively low density. Thus the *Venus* communities of Ford are based on species which are by no means abundant on the grounds in question, and may frequently be absent from the grab samples at a station.

The curves in Fig. 5 would not reach an asymptote until every part of the ground would have been brought up by the sampler. There are always rare species which are scarcely ever taken, and the number of species on a ground may be added to by immigrants from neighbouring grounds.

Some species, seldom taken in grab hauls, are common in trawl or dredge hauls which cover a much larger area of the bottom. An under-water photographic survey of the sea-bed off Plymouth has recently been made by Vevers (1951), who gives figures for the densities of members of the epifauna identified in the photographs. These are in general relatively scarce; thus at position L4, mid-way between the Eddystone and Plymouth breakwater light, the mean density of certain species was: *Asterias rubens*: 1 individual in 10·4 m.^{2}; *Porania pulvillus*: 1 in 83 m.^{2}; *Ophiura texturata*: 1 in 20·8 m.^{2}; *Chlamys opercularis*: 1 in 7·5 m.^{2}; *Turritella communis*: 1 in 83 m.^{2}; *Eupagurus prideauxi*: 1 in 83 m.^{2}; *Hyalinoecia tubicola*: 1 in 83 m.^{2}; *Cellaria* sp.: 1 in 3 m.^{2}.

In the same way certain members of the *infauna* must occur so rarely as seldom to be taken by the sampler.

Variability of samples at a station

The variability in the samples taken at a station was studied by means of twenty successive hauls taken with the double-scoop sampler at B5. Each scoop-full was kept separately and identified. In this way not only could the fauna of successive hauls be compared, but also that in the two adjacent samples taken in each haul.

Details were as follows:

5. xii. 50. Station B5. Marker float anchored at this position, the hauls being made as the ship drifted away from it. After every five hauls the ship steamed back to the mark, so that the samples are positioned along four radiating lines. The extent of drift was estimated after each set of five hauls: (i) 0·25 mile S. from float; (ii) 0·25 mile S.S.E.; (iii) 0·5 mile S.E.; (iv) 0·5 mile S.E.

Wind: light north breeze, freshening during the sampling.

Sieve: 1·2 mm.

The samples were numbered serially—thus nos. 1–10 represent the first five pairs of samples, and nos. 1 and 2 are the two scoop-fulls from the first haul.

TABLE IV. BIGBURY BAY. SPECIES TAKEN IN SUCCESSIVE HAULS OF THE $\frac{1}{20}$ M.^{2} SCOOP-SAMPLER

(The specific identity of the single specimen of *Phacoides* in haul 7 is uncertain. Names in brackets have not been included as data for Figs. 5 and 6.)

Haul no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Amphiura filiformis	7	19	13	18	22	19	11	4	10	10	13	11	15	14	7
?*Sigalion* sp.	..	1	..	1	1	..	..	1	2	2	..	..	..	..	..
Cirratulidae	..	4	..	..	2	..	..	..	..	1	1	..	..	..	3
Montacuta bidentata	..	2	1	9	10	4	6	7	1	8	5	5	6	9	7
Thyasira flexuosa	..	2	3	..	..	1	1	1	1	4	5	2	1	1	4
Cylichna sp.	..	1	..	..	1	1	..	..	..	1	..	..	1	..	..
?*Owenia* sp.	..	..	1	1	..	..	..	..	..	..	..	..	..	..	1
Chlamys opercularis	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..
Nucula nitida	..	..	1	1	..	..	..	..	..	..	1	..	..	..	1
Echinocardium cordatum	..	..	..	2	1	1	1	..	1	..	..	1	2	1	..
Abra sp.	..	..	..	1	2	..	1	1	..	1	..	..	1	..	..
[Amphipoda]	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1
Goniada sp.	..	..	..	..	1	..	1	..	1	..	..	1	..	..	..
[Polychaeta]	..	..	..	..	1	..	..	..	..	..	1	..	2	..	..
Phacoides borealis	..	..	..	..	1	..	1	..	..	..	..	..	..	..	..
Melinna sp.	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..
?*Spisula* sp.	..	..	..	..	..	..	..	..	1	..	..	..	..	..	1
[Nemertinea]	..	..	..	..	..	..	..	..	1	..	..	..	..	1	..
Aphroditidae	..	..	..	..	..	..	..	..	..	..	3	..	..	..	..
Venus striatula	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..
Nassa sp.	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..
Nephthys sp.	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1
Upogebia deltaura	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
Synapta	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1

TABLE V. WHITSAND BAY. SPECIES TAKEN IN SUCCESSIVE HAULS OF THE $\frac{1}{20}$ M.^{2} SCOOP-SAMPLER

(Names in brackets have not been included as data for Figs. 5 and 6. Numbers in brackets indicate that the specific identity is uncertain.)

Haul no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
?*Cardium echinatum*	1	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cochlodesma praetenue	1	..	1	..	2	1	1	..	..	..	..	..	..	1	2	..	..	1	..	1
Mactra corallina	1	..	..	..	..	..	2	..	..	(1)	2	..	1	3	..	..	2	..	..	1
Nephthys sp.	1	2	1	1	..	1	1	1	1	..	..	..	1	1	1	1	..	2	1	1
Magelona papillicornis	8	2	1	5	1	..	6	6	8	3	3	2	4	2	2	2	2	2	..	..
Bathyporeia tenuipes	(1)	..	..	..	..	1	..	1	1	..	..	..	..	..	..	..	..	2	..	..
Urothoë grimaldii var. *poseidonis*	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1
Tellina fabula	..	1	1	..	..	..	..	..	..	..	1	..	1	1	..	..	..	..	..	..
?*Amphiura filiformis*	..	1	1	1	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	..
Natica poliana	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
[Nemertinea]	..	..	1	1	1	..	1	..	..	..	..	..	..	..	..	..	1	..	..	..
[Polychaeta]	..	..	1	2	3	1	4	2	1	1	3	1	2	2	1	1	1	..	2	1
Nucula nitida	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Eteone picta	..	..	..	(1)	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
[Amphipoda]	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Eunicidae	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
?*Owenia* sp.	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
?*Abra* sp.	..	..	..	1	..	..	..	..	1	..	..	..	..	1	..	..	1	..	..	..
Nototropis vedlomensis	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Siphonoecetes colletti	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Leucothoë lilljeborgi	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Dosinia lupinus	..	..	..	..	..	..	(1)	..	..	1	..	..	2	..	..	..	..	1	..	..
[Lamellibranchia]	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..
Bathyporeia elegans	..	..	..	..	..	..	..	..	..	..	1	..	..	1	1	..	1	..	1	1
Echinocardium cordatum	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..
Montacuta ferruginosa	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..
Phyllodocidae	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..
Phascolion strombi	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..
Cirratulidae	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..
Ensis arcuatus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
Leucothoë spinicarpa	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
Cumacea	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
Gari fervensis	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1

TABLE VI. FAUNA OF SUCCESSIVE SAMPLES AT B5, 5 DECEMBER 1950

Sample no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Total
COELENTERATA																																									
[Hydroid]	..	+	..	..	+	..	..	+	..	+	..	+	..	+	+	+	..	..	+	+	..	..	..	..	+	..	..	..	..	..	+	+	..	+	+	..	+	..	..	..	+
Virgularia mirabilis	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Cerianthus lloydi	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	1
Edwardsia sp.	..	..	1	..	..	2	..	2	1	..	1	..	..	..	..	3	1	..	1	2	..	2	1	..	1	2	3	..	1	1	..	..	1	..	1	..	..	1	1	..	29
POLYCHAETA																																									
Lumbriconereis sp.	3	10	7	2	3	8	5	10	3	7	4	3	9	10	2	15	5	15	6	9	10	4	4	4	7	1	7	10	4	9	9	3	5	4	7	10	2	8	4	3	251
Magelona papillicornis	3	1	..	..	7	..	..	1	3	..	4	1	4	..	1	..	..	4	..	4	1	3	4	..	2	1	3	1	1	4	1	..	1	1	..	3	..	1	4	1	65
Magelona cincta	3	..	..	1	1	1	3	3	1	..	3	1	1	4	2	3	1	..	2	..	1	1	2	..	2	1	..	1	..	..	..	..	..	..	1	1	4	2	..	..	46
Chaetopterus variopedatus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	3
Maldanidae	..	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	1	1	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	1	1	..	10
Pectinaria auricoma	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Pectinaria koreni	..	..	..	..	1	..	..	..	1	1	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	6
Amphicteis gunneri	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
[Polychaeta indet.]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GEPHYREA																																									
Phascolion strombi	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
ARTHROPODA																																									
Ampelisca tenuicornis	3	3	1	..	1	1	2	2	..	..	1	..	..	1	2	1	1	4	2	5	..	1	3	3	3	..	4	2	3	4	4	1	2	..	3	2	3	2	1	..	71
Ampelisca brevicornis	..	2	..	..	..	..	..	..	..	..	1	..	..	1	..	..	1	..	1	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	..	..	1	..	9
Ampelisca spinipes	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Bathyporeia tenuipes	..	..	..	..	..	..	..	..	2	1	..	..	..	1	..	..	1	..	..	1	1	1	4	..	..	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	14
Urothoë elegans	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	5
Harpinia antennaria	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	2
Cheirocratus intermedius	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	(1)	..	..	..	..	..	3
Maera othonis	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	1	..	..	..	..	..	2
Acidostoma obesum	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Leucothoë sp.*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Erichthonius brasiliensis	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Nebalia bipes	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1
[Decapoda, callianassid]	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
[Decapod larva]	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
[Mysidacea]	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
['Prawn']	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Galathea sp.	..	..	..	1	..	1	..	..	..	..	..	..	..	1	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1	..	..	..	..	..	..	7
Porcellana longicornis	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	(1)	..	..	..	..	..	..	..	..	..	..	..	..	..	3
Portunus holsatus	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Portunus marmoreus	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Ebalia sp.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
[Pycnogonida]	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Total
MOLLUSCA																																									
Acteon tornatilis	..	..	..	..	..	..	..	..	1	..	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3
Cylichna cylindracea	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	2
Nucula nucleus	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Nucula turgida	..	..	1	..	..	..	1	1	..	..	..	..	..	..	1	..	..	..	..	..	..	1	..	..	1	..	..	1	..	..	1	1	2	1	..	..	..	..	..	..	12
Musculus marmoratus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Chlamys sp.†	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4†
Thyasira flexuosa	..	1	..	..	..	1	1	..	..	2	..	..	..	..	1	..	1	1	..	1	..	1	..	2	..	..	1	..	..	..	..	..	..	..	..	..	1	..	..	2	16
Phacoides borealis†	1	..	..	1	..	..	2	..	1	4	1	1	..	..	..	..	1	..	1	..	1	1	3	..	1	2	..	..	..	2	1	..	..	..	..	1	2	1	1	..	26†
Montacuta ferruginosa	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4
Cyprina islandica	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Cardium echinatum	..	..	..	..	..	..	(1)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	2
Dosinia lupinus	..	1	..	..	1	..	1	1	3	3	1	..	..	..	..	..	2	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	16
Venus ovata	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Venus striatula	..	1	..	..	..	..	..	..	..	..	..	1	1	..	..	1	..	..	..	..	..	..	1	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	6
Venerupis rhomboides	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Abra alba	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	3
Abra prismatica	..	..	..	1	1	..	..	..	1	..	2	..	..	..	..	..	..	..	1	2	1	..	1	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	11
Gari fervensis	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	3
Cultellus pellucidus	1	..	1	..	2	1	..	3	1	3	..	..	2	..	1	..	..	1	..	2	3	..	1	1	2	1	1	1	1	..	..	1	1	1	..	3	1	2	1	1	40
Hiatella arctica	..	..	..	..	..	..	..	2	..	1	..	..	..	..	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	6
Cochlodesma praetenue	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	3
Lyonsia norwegica	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
POLYZOA																																									
Cellaria sp.	..	..	..	..	..	+	..	..	..	+	..	+	..	+	..	+	..	+	−	+	+	..	..	..	..	..	+	..	+	..	+	..	..	..	+	..	..	..	..	..	+
ECHINODERMATA																																									
Ophiothrix fragilis	..	..	..	..	..	..	..	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Amphiura filiformis	1	..	1	1	2	..	..	..	..	..	2	2	..	1	..	6	1	1	..	1	..	1	1	..	6	2	1	3	1	2	..	1	..	..	1	..	..	..	1	2	41
Acrocnida brachiata	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1
Ophiura affinis	..	..	1	..	..	..	(1)	5	..	2	..	..	3	3	1	2	1	2	1	1	2	..	..	1	4	..	..	2	3	3	4	..	..	..	..	2	1	..	1	2	48
Ophiura texturata	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Echinocyamus pusillus	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	..	..	1	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4
Echinocardium cordatum	..	1	..	..	..	..	..	(1)	..	..	..	(1)	..	..	(1)	..	1	..	..	..	(2)	..	..	(1)	..	..	(1)	(1)	..	(1)	..	..	(1)	..	..	..	(2)	1, (3)	..	..	18
Labidoplax digitata	..	..	..	1	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	1	1	..	..	..	..	..	6
TUNICATA																																									
Ascidia mentula	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1
Ascidiella aspersa	..	..	..	..	..	..	..	1	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3
Diplosoma listerianum	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
ENTEROPNEUSTA																																									
Glossobalanus sarniensis	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	..	..	..	..	..	..	..	..	..	2
Total species taken	9	17	21	25	28	31	32	39	42	43	44	44	47	48	50	52	52	53	54	55	55	55	57	57	57	57	58	60	61	61	61	62	62	62	62	62	62	63	63	64	

* See p. 25.

† See corrections at foot of Table **XXIII**. The totals in the right-hand column are corrected.

Names in brackets have not been used as data for Figs. 5 and 6. Numbers in brackets indicate that the specific identity is uncertain.

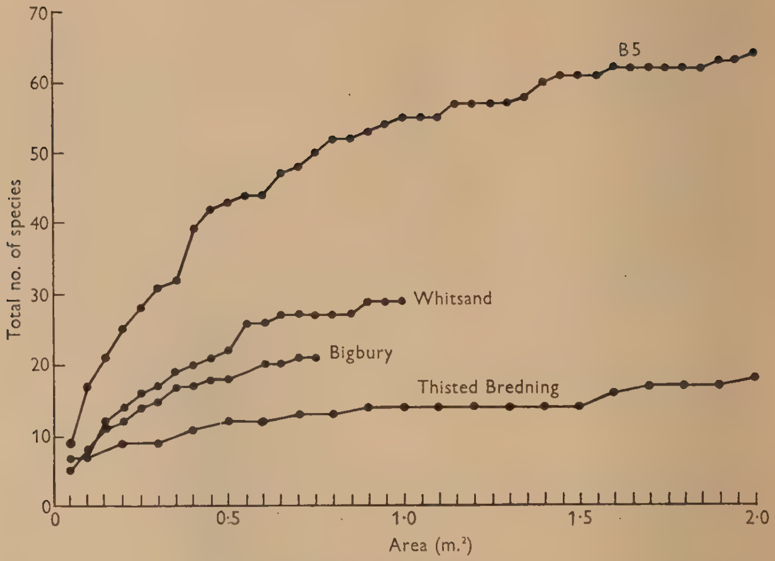

Fig. 5. Cumulative curves showing the number of species taken in successive samples of the sea-bed in four localities.

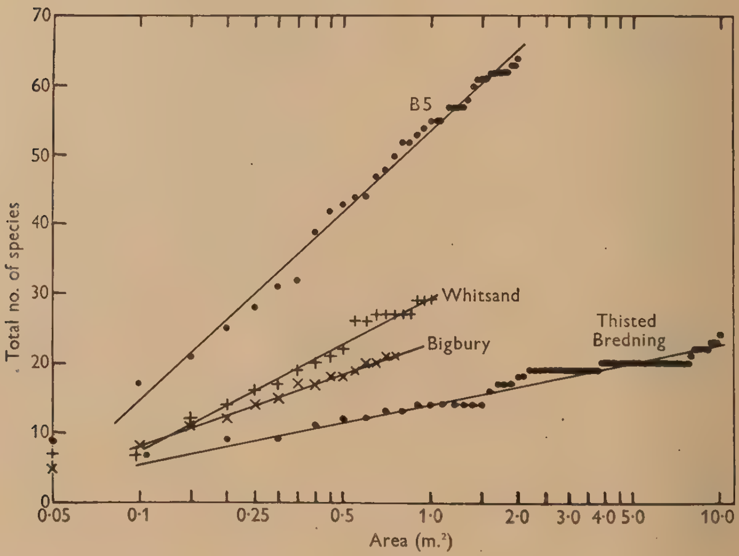

Fig. 6. The same samples as in Fig. 5 plotted with area on a log scale. Note that the samples from Thisted Bredning are each twice the area of those at the other stations.

The fauna is shown in Table VI. Most of the species occur at rather low densities, and there are no signs of gross aggregation or that the samples pass through more than one 'community'. On the other hand, there seems to be almost as much variation between the pairs of samples from one haul as between samples in successive hauls.

A statistical examination has been made of the distribution of the common species. The variance for each species has been analysed as follows, that for *Ophiura affinis* being chosen as an example:

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio	Probability
Successive hauls	37·4	19	1·97	1·64	0·05–0·02
Pairs of samples	34·9	19	1·84	1·53	—
Residual	0·1	1	0·10	0·08	—
Total	72·4	39	1·86	1·55	0·02–0·01

The variance between successive hauls is a measure of the large-scale patchiness of the fauna, there being perhaps 100 or 200 yards distance between each haul. The variance between the pairs of samples taken in each haul is a measure of distribution on a small scale, and there is a residual variance due to any differential sampling by the two scoops. Since no record was made of which of each pair of samples came from either scoop, this residual variance should not be significant. Analyses for the more frequent species to be compared against the expected random variance given by the Poisson distribution are given in Table VII.

As might be expected the variance between hauls tends to be greater, and therefore more frequently significant, than that between the pairs of samples from a single haul. An exception is *Magelona papillicornis* where the variance between pairs of samples is significant, but that between hauls is not.

A measure of the degree of patchiness is given by Fisher's 'coefficient of dispersion' (used by Holme, 1950*b*), which equals the Variance Ratio. This tends to unity in a randomly distributed population, to less than unity in an evenly distributed population, and to more than unity if there is aggregation. Treating the forty samples separately, the coefficients have been calculated for the same species (Table VII). Although significantly high for six species, the coefficients do not indicate any great degree of aggregation. *Edwardsia*, *Ampelisca tenuicornis*, *Thyasira*, and *Cultellus* appear to have a random distribution.

On this ground the fauna is sufficiently well dispersed for repeatable results to be obtained; but closer inshore the grounds are so patchy that this is not possible.

Seasonal changes

Most of the collections were made in the summer months, and no account has been taken of any seasonal variation in the fauna.

Blegvad (1925) found a seasonal variation in the *Macoma* and *Abra* communities of the enclosed Danish Fjords, but in more open waters the variation was less marked. Cold winters seem to play a large part in reducing populations in shallow water. Steven (1930), in a grab survey of an offshore area at Plymouth, found no marked seasonal changes in the fauna, and a recent paper by Jones (1952) confirms this view from observations made off the Cumberland coast, in the Irish Sea.

TABLE VII. COEFFICIENTS OF DISPERSION AND ANALYSIS OF VARIANCE OF CERTAIN SPECIES FROM B5 (SEE TABLE VI)

Significant coefficients of dispersion are in bold type (test of significance 1 ± 0·4586). Only significant probabilities are given.

	Coefficient of dispersion	Probability			
		Hauls	Scoops	Residual	Total
Edwardsia sp.	1·060	..	..	..	..
Lumbriconereis sp.	**1·888**	..	0·01–0·001	0·02–0·01	<0·001
Magelona papillicornis	**1·852**	..	<0·001	..	<0·001
Magelona cincta	1·273	0·05–0·02	..	..	..
Ampelisca tenuicornis	1·112	..	..	..	..
Bathyporeia tenuipes	**1·692**	0·02–0·01	..	..	0·01–0·001
Thyasira flexuosa	1·000	..	..	..	..
Dosinia lupinus	**1·513**	<0·001	..	..	0·05–0·02
Cultellus pellucidus	0·872	..	..	..	..
Amphiura filiformis	**1·926**	0·01–0·001	0·05–0·02	..	<0·001
Ophiura affinis	**1·547**	0·05–0·02	..	..	0·02–0·01

Some seasonal changes must occur coinciding with the settling and growth of young, but the larger benthic invertebrates are usually considered to have a life-span of several years and fluctuations in total population density are thus reduced.

Most of the populations studied were composed of large numbers of species, few of which occurred in abundance, and this diversity should minimize the effects of seasonal and annual fluctuations.

Sieving

The samples were washed through a gauze sieve of 2·2 mm. aperture on board ship, and the sievings kept for examination in the laboratory. The use of a sieve to separate the fauna from the sediment necessarily results in a lower size limit to the animals collected. With animals of a definite size and shape, e.g. Mollusca, the proportion of animals passing through the sieve is dependent on the size of the individuals, but many worms actively crawl through the meshes and quite large individuals may be lost during a prolonged sieving. It is advisable to wash out the contents of the sieve into a jar as often as possible to minimize such losses.

While a finer meshed sieve would undoubtedly retain many more small amphipods, worms, etc., the 2·2 mm. sieve was used because of the high percentage of coarse particles at some stations which soon clog a finer sieve.

TABLE VIII. NUMBERS AND WEIGHTS OF ANIMALS AT C4 (7 NOVEMBER 1950) AFTER WASHING SAMPLE THROUGH FIRST THE 2·2 MM. AND THEN THE 1·2 MM. SIEVE

(Numbers in brackets indicate doubtful identity.)

	2·2 mm.		1·2 mm.	
	No.	Dry weight (g.)	No.	Dry weight (g.)
Hydroid	+	0·003	..	..
Anemone	1	0·083	..	..
Polychaeta	6	0·085	+	0·31
Diastylis laevis	..	..	(5)	0·002
Isopoda	..	..	1	
Ampelisca tenuicornis	..	..	5	
Ampelisca diadema	..	..	1	
Bathyporeia tenuipes	..	..	6	
Harpinia antennaria	..	..	2	
Photis longicaudata	1	<0·001	..	
Pycnogonida	..	..	4	
Nucula turgida	..	..	8	0·022
Thyasira flexuosa	4	0·022	7	
Dosinia lupinus	2	0·003	(1)	
Venus striatula	1	0·001	..	
Mysia undata	2	0·16	..	
Abra alba	1	0·006	..	
Abra prismatica	1	0·022	(7)	
Gari fervensis	..	..	3	
Cultellus pellucidus	1	0·042	1	
Hiatella arctica	1	0·001	..	
Thracia sp.	4	0·011	10	
Cellaria sp.	+	0·005	..	..
Amphiura filiformis	1	0·001	7	0·001
Ophiura affinis	1	0·001	(1)	
Echinocyamus pusillus	..	..	1	
Cucumaria elongata	1	0·073	..	..
Total		0·52		0·34

The same mesh was employed at all stations to ensure a uniformity in sampling technique. Petersen & Boysen Jensen (1911) used a sieve of 1·5 mm. aperture for sieving deposits consisting mainly of fine particles, and where the soil is fine no coarser mesh should be employed.

The use of a series of sieves, such as the *Challenger* series, has not been found practicable, as the smaller meshed sieves are liable to clog and overflow, and some of the smaller animals are thereby lost.

On 7 November 1950 station C4 was sampled a second time. The sample, which was of fine sand, was sieved through first a 2·2 mm. and then a 1·2 mm.

sieve. The fauna taken in each sieve is shown in Table VIII. In this sample the coarse sieve failed to retain a high proportion of the individuals. Of the total dry weight, nearly two-fifths passed the 2·2 mm. sieve and was retained by the finer mesh. At most stations, however, the *relative* losses are probably not as high as this. In the twenty stations worked, 64·4% of the total dry weight was made up of fairly large individuals, each weighing more than 0·2 g. dry (p. 38), whereas at this station there were no individuals of this size. The previous samples taken at C4 had given a total of 5·7 g. per $\frac{1}{2}$ m.^{2} (p. 35), and the second series has an exceptionally low dry weight. If we take the losses at each station through using the 2·2 mm. sieve as about 0·4–0·5 g., or say 10% of the total taken, a reasonable correction can be made.

The animals lost through the 2·2 mm. sieve correspond to the 'small macrobenthos' of Mare (1942). In the Rame mud a mile or two north of A2 she found that small polychaetes, etc., retained on the 1 mm. sieve represented about 30·6% of the total (fresh) weight of macrobenthos. Polychaetes are abundant in the Rame mud, and a smaller percentage would therefore be expected for other grounds.

Biomass estimations

Any estimate of the productivity of a community must start with an assessment of the 'standing crop', that is, the quantity of living tissue present at any one time. Owing to the very different sizes of species and individuals, an estimate of their numerical density is insufficient, and comparisons are best made in terms of weight.

The biomass may be expressed either as: (i) the 'fresh weight', i.e. the weight of fresh tissue after surface moisture has been blotted off, the weight of mollusc shells may or may not be included; (ii) the dry weight, shells usually being excluded from the total.

In this investigation the dry weight of the specimens has been determined, and is here defined as the weight of the residue obtained after evaporation at 110° C. to a (more or less) constant weight, surface moisture having previously been removed. The weight of all substances soluble in dilute hydrochloric acid has been excluded from the total. The dry weight thus includes chitinous or similar non-calcareous skeletal structures, inorganic salts in the blood and tissues, and gut contents (except in *Echinocardium*), but excludes all calcareous structures.

The animals were usually preserved in formalin on board ship, but were transferred to and stored in 70% alcohol within a day or two. The 'alcohol weight' was determined from preserved specimens by blotting off surface moisture with filter-paper, and weighing as quickly as possible to the nearest centigram.

During preservation certain constituents of the tissues dissolve out into the alcohol. To find the relation of dry weight to alcohol weight two or three specimens of certain species were preserved in 70% alcohol for at least a week. The preserved animals were then dried at 110° C. overnight and to the dry weight was added that of the residue obtained from evaporating the alcohol.

The dry weight has been calculated as a percentage of the alcohol weight for a number of species (Table IX). For the rest a value of 27·72% has been taken, being the mean of determinations for species not having a calcareous skeleton. It was more convenient to measure the size rather than the weight of some species, and by a series of determinations of dry weight a factor was obtained for converting a size measurement directly into terms of dry weight.

TABLE IX. DRY WEIGHT EXPRESSED AS A PERCENTAGE OF ALCOHOL WEIGHT

(Species marked with an asterisk were treated with acid before the dry weight was determined. Figures in italics are assumed or approximate percentages. The figure given for Amphipoda is derived from Molander (1928), and allows for matter dissolved out by the alcohol. It appears to be too low, but in any case the 'biomass' of the amphipod population is extremely small.)

COELENTERATA	
Hydroids and anemones	*27·72*
*Alcyonium**	*10·00*
POLYCHAETA	
Chaetopterus	25·24
All other spp.	*27·72*
CRUSTACEA	
Amphipoda etc.	4·12
*Callianassa** (and other Macrura)	19·83
*Gonoplax** (and other Brachyura)	24·20
MOLLUSCA	
Cyprina islandica	28·14
Solecurtus chamasolen	31·05
Tellina crassa	25·74
All other spp.	*27·72*
POLYZOA	
*Cellaria**	3·43
ECHINODERMATA	
*Ophiothrix fragilis**	14·56
*Amphiura** (and other Ophiuroidea)	5·54
*Echinocardium** (and *Echinocyamus*)	2·29
*Cucumaria**	14·66
*Leptosynapta** (and *Labidoplax*)	24·16
CEPHALOCHORDATA	
Amphioxus	28·41

(The conversion factors from alcohol to dry weight differ from those given by Petersen & Boysen Jensen (1911, p. 52). They apparently added the weight of the material, obtained from evaporating the alcohol, to the total *after* applying their conversion factor. In the present investigations the quantity dissolved in the alcohol is allowed for in the conversion factor.)

A number of observations were made to assess the validity of these techniques:

(i) It was found that after about a week most of the soluble substances had dissolved out into the alcohol. A change to fresh alcohol did not greatly increase the total amount dissolved out.

(ii) Provided that a reasonable quantity of preservative was used, the amount of dry matter dissolved out of the tissues was not dependent on the relative volumes of the animals and the spirit.

(iii) About half as much again was dissolved out if 96 % alcohol was used in preservation.

(iv) Weight of gut contents. Where a species contains much sand in the gut, a considerable error might be introduced in dry-weight determinations. A number of alcohol-preserved animals were weighed before and after ignition. The residue after ignition is ash, together with any sand from the gut. The weights after ignition, expressed as percentages of the alcohol weight, were:

Chaetopterus variopedatus	(Polychaeta)	1·09 %
Solecurtus chamasolen	(Lamellibranchia)	1·00 %
Abra alba	(Lamellibranchia)	7·43 %
Ophelia bicornis	(Polychaeta)	23·93 %

Miscellaneous small worms from bottom-sampler hauls: 2·23 %.

Taking ash as 1 % of the alcohol weight (i.e. assuming that the *Chaetopterus* and *Solecurtus* residue was entirely 'organic ash'), the rest is sand. *Ophelia* is a deposit-feeding worm in which the gut can be seen to be full of sand, and represents a maximum figure. *Abra* feeds on detritus collected from the soil surface, and might be expected to take in some sand with the food. *Chaetopterus* and *Solecurtus* both draw their food from material in suspension just off the bottom, and so probably take in very little sand under normal conditions.

An estimate that the *alcohol* weights include 5 % of sand in the guts of animals seems a reasonable overall estimate.

(v) Calcareous skeletons. The shells of molluscs were removed before weighing, but with other forms it was necessary to determine an alcohol weight which included the skeleton. To determine the dry weight, the skeleton was then dissolved by placing the animal in dilute hydrochloric acid, the mixture being filtered, washed with distilled water, dried and weighed.

Treatment with dilute acid did not appear to release into solution appreciable quantities of non-calcareous substances within the tissues. Treatment of a non-calcareous organism (the polychaete *Nereis*) with acid did not alter the alcohol weight/dry weight ratio, but it was thought that where an animal had calcareous spicules embedded in the tissues, some loss might result from the mechanical break-up of cells due to formation of bubbles of carbon dioxide.

For non-calcareous animals there is a fairly constant proportion of the total dry weight found in solution in the alcohol. If acid treatment after determination of the alcohol weight resulted in the loss of material into the acid solution, a resultant change in the ratio of the weight of dried animal tissue to total dry weight would occur. In six species which were not acid treated, the percentages of the total dry weight found in the dried preserved animal were: *Nereis diversicolor*, 59·04; *Amphioxus lanceolatus*, 68·33; *Solecurtus chamasolen*, 64·78; *Tellina crassa*, 51·13; *Chaetopterus variopedatus*, 56·53; *Cyprina islandica*, 73·69; Mean: 62·25. In four species in which the skeleton was dissolved in acid, the percentages were: *Ophiothrix fragilis*, 50·06; *Callianassa subterranea*, 58·28; *Gonoplax rhomboides*, 54·90; *Cucumaria elongata*, 69·55; Mean: 58·20. There is therefore no evidence of any considerable loss due to acid treatment.

In *Echinocardium cordatum* the alcohol weight is made up largely of both calcium carbonate in the skeleton and sand in the gut, plus a certain amount of sea water inside the test. After acid treatment, as above, the residue was ignited and the percentage of sand calculated. Allowance for the sand content of the gut has been made in the factor given in Table IX.

Nomenclature

Nomenclature is that of the *Plymouth Marine Fauna* (Marine Biological Association, 1931), except for the Mollusca, which are named as in Winckworth's (1932) list of British marine Mollusca. The following species are not in the *Plymouth Marine Fauna*:

POLYCHAETA

Sigalion mathildae Audouin & M.-Edwards* (see Fauvel, 1923).
Magelona cincta (Ehlers) (see Mare, 1942, p. 542).

AMPHIPODA

Ampelisca diadema (A. Costa) (see Chevreux & Fage, 1925).
Bathyporeia elegans Watkin (see Watkin, 1938).
B. tenuipes Meinert (see Watkin, 1938).
Urothoë grimaldii Chevreux var. *poseidonis* Reibisch (see Chevreux & Fage, 1925, p. 100 as var. *inermis*).
Urothoë elegans Bate (see Chevreux & Fage, 1925).
Leucothoë lilljeborgi Boeck (see Chevreux & Fage, 1925).
Leucothoë sp. (the undescribed species mentioned in Spooner, 1950, p. 249).

LAMELLIBRANCHIA

*Cochlodesma praetenue** (Montagu) (see Forbes & Hanley, 1853).

ENTEROPNEUSTA

Glossobalanus sarniensis (Koehler)* (see Koehler, 1886).

Species marked with an asterisk (*) are first records for the Plymouth area.

DETAILS OF STATIONS AND COLLECTIONS

Station A 1

50° 19′ 40″ N., 4° 14′ 45″ W. Clean sand (Table I, Fig. 2). Depth *c.* 15 m. (at all stations the depth is given from soundings on the Admiralty Chart of the area). 18. x. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm. *Ship anchored.*

This station is slightly out of line with the other stations to avoid a 'dumping ground'. The fauna (Table X) is similar to that given in Table V, taken at a position about half a mile distant.

The bottom in this part of Whitsand Bay is very patchy, areas of rock or gravel occurring in places. The small polychaete *Magelona papillicornis* is common, and some specimens were probably lost through the sieve. *M. cincta*, a rather larger worm, occurs here and at several other stations. It was first recorded from Plymouth by Mare (1942); it is probably not new to the area, and may have been confused with *M. papillicornis* in earlier records (D. P. Wilson, personal communication).

The total dry weight at this station is 1·5 g./½ m.², which is lower than for most stations in the survey. There were no large individuals apart from five specimens of *Echinocardium*.

TABLE X. STATION A 1. NUMBERS AND DRY WEIGHTS PER ½ M.²

(Numbers in brackets are approximate. *j*: young individuals.)

	No.	Weight		No.	Weight
Nemertinea indet.	(2)	0·50	*Portunus* sp.	1*j*	0·025
Sigalion mathildae	2		*Natica poliana*	2*j*	0·004
Aphroditidae	1		*Philine* sp.	1*j*	0·007
Phyllodocidae	1*j*		*Nucula turgida*	1*j*	0·001
Nephthys sp.	2		*Dosinia lupinus*	3*j*	0·004
Glycera sp.	1		*Tellina fabula*	5	0·037
Magelona papillicornis	(65)		*Ensis ensis*	1*j*	0·013
Magelona cincta	1		*Ensis arcuatus*	1*j*	0·011
Spionidae	1		*Mactra corallina*	1	0·069
Polychaeta indet.	1		*Cellaria* sp.	+	< 0·001
Diastylis laevis	1	< 0·001	*Ophiura affinis*	1	0·002
Ampelisca brevicornis	1		*Echinocardium cordatum*	5	0·87
Gastrosaccus sanctus	2		Total dry weight		1·5

TABLE XI. STATION A2. NUMBERS AND DRY WEIGHTS PER ½ M.²

(Where it has been necessary to assume the weight of an individual, either because it was badly fragmented or because only part of it was taken, the figures are italicized.)

	No.	Weight		No.	Weight
Hydroid	+	0·092	*Alpheus ruber*	2	0·93
Anemones	2	0·73	*Callianassa subterranea*	1	0·025
Eunice harassi	1	0·99	*Portunus depurator*	1	0·42
Maldanidae	1		*Chlamys* sp.	1*j*	0·001
Melinna palmata	(120)		*Cucumaria elongata*	2	0·67
Polychaeta indet.	1		*Leptosynapta inhaerens*	2	*0·97*
Phyllochaetopterus tubes	(4)	..			
Scalpellum scalpellum	1	0·011	Total dry weight		4·8

Station A2

50° 17′ N., 4° 15′ W. Very fine muddy sand (Table I, Fig. 2). Depth: 46 m. 26. vi. 50. Area: 0·5 m.². Sieve: 2·2 mm. Ship drifting at this and at all subsequent stations.

This station on the southern edge of the 'Rame mud' is characterized by the abundance of *Melinna palmata*, and the presence of *Alpheus ruber*, *Callianassa subterranea*, *Cucumaria elongata* and *Leptosynapta inhaerens*, which together constitute a large proportion of the total dry weight (Table XI). Lamellibranchs are scarce in this deposit, the only species commonly taken in dredgings being *Solecurtus chamasolen*.

The station is somewhat to the south of stations worked in the Rame mud by Ford (1923) and Mare (1942). The latter estimated a total of over 100 g./m.² of fresh tissue in the Rame deposit (see p. 43).

Station A3

50° 15′ N., 4° 15′ W. Hauls nos. 1, 2 and 6 on clean medium-grade sand, hauls 3–5 on mixed muddy sand and gravel. Depth: 51 m. 26. vi. 50. Area: 0·6 m.². Sieve: 2·2 mm.

The sediment at this station is very patchy, two distinct grades of deposit being taken in the sampler. The first is a medium-grade sand with less than

1 % over 2 mm., and 1·5 % silt; the second a muddy gravel with 28·9 % over 2 mm. and 7 % silt (Table I and Fig. 2). The fauna (Table XII) in the different hauls probably varied somewhat, but is characteristic of the muddy sand grounds inside the Eddystone, where typical species are: the polychaetes *Nephthys* sp., *Eunice harassi*, *Hyalinoecia bilineata*, *Lumbriconereis* sp., burrowing prawns *Upogebia deltaura* and *U. stellata*, the lamellibranchs *Dosinia lupinus*, *Cultellus pellucidus* and *Solecurtus chamasolen*, and the heart-urchin *Echinocardium cordatum*. The polyzoan *Cellaria* occurs in clumps and was quite abundant in some samples. Three species of *Cellaria* are recorded in the *Plymouth Marine Fauna* (Marine Biological Association, 1931), but specific identifications were not made in this survey.

Polychaeta make up about half the total dry weight. *Cellaria* contributes only 0·5 g. to the total; its fresh weight being largely composed of calcium carbonate in the skeleton. The total dry weight, 4·0 g./½ m.², is rather below the average for the area.

TABLE XII. STATION A3. NUMBERS AND WEIGHTS PER 0·6 M.²

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·40	*Phyllochaetopterus* tubes	4	..
Anemone	1	0·53	*Owenia* tube	1	..
Edwardsia sp.	2*j*	0·083	*Ampelisca spinipes*	4	0·011
Nemertinea indet.	1	2·0	*Ampelisca tenuicornis*	2	
Nephthys sp.	3		*Upogebia stellata*	1	0·34
Glycera sp.	2		*Modiolus phaseolinus*	1	0·007
Eunice harassi	1		*Dosinia lupinus*	1*j*	0·001
Nematonereis unicornis	1		*Cultellus pellucidus*	2	0·050
Hyalinoecia bilineata	1		*Cellaria* sp.	+	0·51
Lumbriconereis sp.	(22)		*Amphiura filiformis*	1	0·014
Maldanidae	1		*Amphipholis squamata*	3	0·003
Capitellidae	5		*Ascidiella aspersa*	1	0·007
?*Jasmineira elegans*	2		Total dry weight (0·6 m.²)		4·0
Terebellidae	1				
Polychaeta indet.	4				

Station A4

50° 13′ N., 4° 15′ W. Muddy sand with small stones and shell fragments. Depth: 55 m. 18. x. 50. Area: 0·5 m.². Sieve: 2·2 mm.

As at A3, there was considerable variation in the grade of deposit brought up in successive hauls. Two types of sediment were taken, their grades corresponding closely to those at A3 (Table 1 and Fig. 2).

The fauna (Table XIII) is similar to that at the previous station. Polychaete worms are common and make up over a quarter of the total weight. Three specimens of *Upogebia deltaura* were taken, whereas *U. stellata* was taken at A3.

The total dry weight is 6·9 g., largely made up of Polychaeta, *Upogebia*, and the crab *Portunus depurator*.

Station A5

50° 11′ N., 4° 16′ 15″ W. (Eddystone bearing 150° T., 0·5 miles.) Shell gravel (Table I, Fig. 2). Depth: *c.* 42 m. 21. vii. 50. Area: 0·5 m.². Sieve: 2·2 mm.

This station is situated on the Eddystone shell gravel where dredgings are frequently made for *Amphioxus*. This position was worked to avoid a possibly rocky ground on the east side of the reef, and since an area was selected where the fauna was known to be quite rich a slight bias is given to the results as a whole, since all but one of the other stations were selected at definite intervals irrespective of the possible nature of the ground.

Since the position is so close to the Eddystone Lighthouse, it is possible to obtain an accurate position fix, but the ship soon drifts off the ground, and while making hauls with the bottom-sampler it was necessary to steam back to the original position after two or three hauls.

TABLE XIII. STATION A4. NUMBERS AND WEIGHTS PER 0·5 M.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·16	*Galathea* sp.	3*j*	0·019
Anemone	1	0·049	*Upogebia deltaura*	1, 2*j*	1·6
Edwardsia sp.	3*j*	0·11	*Portunus depurator*	1	2·0
Glycera sp.	7	1·9	Pycnogonida	1	<0·001
Hyalinoecia bilineata	22		*Cylichna cylindracea*	4	0·011
Onuphis conchylega	4		*Lepton squamosum*	1	0·003
Lumbriconereis sp.	4		*Phacoides borealis*	1	0·040
?*Owenia* sp.	(16)		?*Cardium echinatum*	1*j*	0·010
Pectinaria sp.	3*j*		*Solecurtus chamasolen*	1	0·075
Maldanidae	1		*Cellaria* sp.	+	0·046
Terebellidae	2		*Alcyonidium* sp.	6	0·43
Sabellidae	1		*Ophiura affinis*	1	0·002
Polychaeta indet.	+		*Echinocyamus pusillus*	2	<0·001
Phyllochaetopterus tubes	+	..	*Labidoplax digitata*	1	*0·48*
'Prawn'	1*j*	0·006	Total dry weight		6·9

TABLE XIV. STATION A5. NUMBERS AND WEIGHTS PER 0·5 M.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Porifera indet.	2*j*	<0·001	*Venus fasciata*	1	0·078
Hydroid	+	0·014	*Tellina crassa*	1	0·89
Aphroditidae	1	0·33	*Gari tellinella*	1	0·055
Glycera sp.	1		*Solecurtus scopula*	1	1·5
Petta pusilla	1		?*Lutraria* sp. (siphons)	1	5·5
Terebellidae	1		*Cellaria* sp.	+	0·012
Polychaeta indet.	3		*Ophiothrix fragilis*	7	1·4
Phascolosoma sp.	1		*Echinocyamus pusillus*	5	0·003
Ampelisca spinipes	1	0·004	*Amphioxus lanceolatus*	7	0·35
Conilera cylindracea	4	0·030	(Egg case of skate)	1	..
Ebalia tuberosa	1	0·13			
Cardium scabrum	1*j*	0·011	Total dry weight		10·3

Smith (1932) has studied the fauna and deposits around the Eddystone in some detail. The species characteristic of the shell-gravel are species of the 'SpVf' (*Spatangus purpureus-Venus fasciata*) communities of Ford (1923).

The siphons of a large lamellibranch, probably *Lutraria*, were taken (Table XIV). Ford records specimens of *L. magna* from shell-gravel in Plymouth Sound, so these may be of the same species. The animal was judged to be adult, and was assumed to have an alcohol weight of 20 g. (5·5 g. dry).

Polychaete worms form only a small fraction of the total dry weight, which is largely made up of moderate-sized lamellibranchs, and the *Lutraria*. *Ophiothrix* seems to be patchily distributed on this ground.

For stations A6 and A7 see pp. 32–33.

Station B1

50° 18′ N., 4° 10′ W. Shell gravel with coal and clinker. Depth: 31 m. 26. vi. 50. Area: 0·1 m.^{2}. Sieve: 2·2 mm.

Three hauls were made, one of which brought up a sample of gravel (Table I); the other two were evidently on a rocky bottom.

A single specimen of *Tellina crassa* was taken with a dry weight of 0·64 g. This is equivalent to 3·2 g./$\frac{1}{2}$ m.^{2}.

TABLE XV. STATION B2. NUMBERS AND WEIGHTS PER $\frac{1}{2}$ M.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·078	*Phyllochaetopterus* tubes	+	..
Alcyonium sp.	1	0·018	*Owenia* tubes	10	..
Nemertinea indet.	1	1·4	*Ampelisca spinipes*	1	0·005
Glycera sp.	2		*Callianassa subterranea*	1*j*	0·013
Marphysa belli	1		*Upogebia deltaura*	2	3·5
Cirratulidae	1		*Mysia undata*	1	0·057
Notomastus latericeus	2		*Abra alba*	1	0·018
Amphicteis gunneri	10		*Solecurtus chamasolen*	1, 10*j**	1·1
?*Melinna* sp.	11		*Cellaria* sp.	+	0·098
Maldanidae	2		*Cucumaria elongata*	1	0·081
Myxicola infundibulum	1		Total dry weight		6·4

* One small specimen in a $\frac{1}{10}$ subsample.

Station B2

50° 16′ N., 4° 10′ W. Fine muddy sand with small stones. Depth: 48 m. 18. v. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm. A marker float was achored close to the position of the first haul, and the total drift during five hauls was found to be about 150 yards (137 m.).

The percentage of coarse material was high (Table I), 27% being over 2 mm., and there was a fairly high percentage of silt (8·6%) which resulted in the presence of such species as *Notomastus*, *Myxicola*, *Solecurtus chamasolen* and *Cucumaria elongata* (Table XV). Two species of burrowing prawn, *Callianassa subterranea* and *Upogebia deltaura*, were taken.

The total weight of 6·4 g. is largely made up of two specimens of *Upogebia* and one large and several small *Solecurtus*.

Station B3

50° 14′ N., 4° 10′ W. Muddy sand. Depth: 53 m. 18. v. 50. Area: 0·5 m.^{2}. Sieve: 1·2 mm.

The soil was finer and less muddy (4·4% silt) than at B2 (Table I). Some of the same species occurred (Table XVI) (*Abra alba*, *Solecurtus chamasolen*), but the difference in soil is reflected in the presence of species characteristic of slightly muddy sand: *Cultellus*, *Echinocardium* and *Labidoplax*.

The deposit was sieved through a finer sieve than usual, and this retained a greater number of polychaete worms and small Crustacea. They appear to have little effect on the total biomass, however, the dry weight for polychaetes being of the same order as at similar stations where the 2·2 mm. sieve was employed (A3, A4 and B2), and the weight of the small Crustacea is negligible.

The large sabellarian worm *Pallasia murata* contributes about a quarter to the weight of polychaetes. Much of the total dry weight is made up of large individuals—*Solecurtus*, *Echinocardium* and *Labidoplax*.

For stations B4–B7 see pp. 29–36.

Station C1

50° 17′ N., 4° 5′ W. Fine gravel of shell fragments and small stones. Depth: 37 m. 7. xi. 50. Area: 0·5 m.^{2}. Sieve 2·2 mm.

The soil at this station was rather variable, some hauls being rather muddy and others quite clean (Table I).

The fauna is rather poor (Table XVII), the total dry weight being only 1·3 g.

Station C2

50° 15′ N., 4° 5′ W. Muddy sand with a few stones (Table I). Depth: 46 m. 7. xi. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

The fauna (Table XVIII) is typical of muddy sand, but is rather poor. The occurrence of *Lepton squamosum*, a small lamellibranch commensal with *Upogebia*, is of interest.

Apart from *Labidoplax*, there are no individuals of any size, and the total dry weight is only 2·5 g.

Station C3

50° 13′ N., 4° 5′ W. Muddy sand with a few stones (Table I). Depth: 46 m. 7. xi. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

The bottom at this station is evidently rocky with pockets of sediment. A single core sample (Table II) showed no sediment overlying the rock. The fauna (Table XIX) is rather poor, except for fifteen specimens of *Ophiothrix*, which were all or nearly all taken in a single haul of the sampler.

The total dry weight, 4·5 g., is rather below the average.

Fauna of sandy grounds around the Eddystone

At the stations so far described there is much variation in both grades of deposit and fauna, but at certain stations farther offshore, to the south and east of the Eddystone, there is a marked uniformity in grade of soil and fauna.

As already shown (pp. 14–19), the fauna in successive hauls at B5 does not vary greatly, being little more patchy than would be expected from a random distribution. The same uniformity extends over a much larger area, a very similar fauna being found at stations A6, A7, B4, B5, B6, B7, C4, C5 and C6.

Table XVI. Station B3. Numbers and Weights per $\frac{1}{2}$ m.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·13	*Diastylis laevis*	1	0·002
Aphroditidae	3	1·4	*Ampelisca diadema*	2	
Nephthys sp.	(8)		*Ampelisca tenuicornis*	4	
Eunice harassi	2		*Maera othonis*	2	
Lumbriconereis sp.	2, 8*j*		*Photis longicaudata*	2	
Scoloplos armiger	1		*Abra alba*	1*j*	0·001
Magelona cincta	1		*Solecurtus chamasolen*	3	2·4
Cirratulidae	6		*Cultellus pellucidus*	2*j*	0·003
Ammotrypane aulogaster	1		*Cellaria* sp.	+	0·51
Pallasia murata	1		Polyzoa indet.	+	0·009
Polychaeta and Nemertinea indet.	(40)		*Acrocnida brachiata*	1	0·067
Phascolion strombi	2		*Echinocardium cordatum*	2	0·57
Chaetopterus tube	1	..	*Thyone raphanus*	1	0·14
Phyllochaetopterus tubes	+	..	*Labidoplax digitata*	1	0·48
Scalpellum scalpellum	3*j*	0·007	Total dry weight		5·7

Table XVII. Station C1. Numbers and Weights per $\frac{1}{2}$ m.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Nephthys sp.	1	0·56	*Upogebia deltaura*	2	0·16
Glycera sp.	3		*Chlamys opercularis*	2	0·49
Hyalinoecia bilineata	19		*Cellaria* sp.	+	0·004
Nerine sp.	1		*Echinocyamus pusillus*	22	0·011
Polychaeta indet.	(60)		*Thyone fusus*	(10)*	0·073
Ampelisca spinipes	3	0·003			
Maera othonis	1		Total dry weight		1·3

* One specimen in a $\frac{1}{10}$ subsample.

Table XVIII. Station C2. Numbers and Weights per $\frac{1}{2}$ m.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·014	*Galathea* sp.	1*j*	0·004
Edwardsia sp.	1*j*	0·014	*Upogebia deltaura*	1*j*	0·047
Anemone indet.	1	0·20	*Lepton squamosum*	1	0·001
Nephthys sp.	(8)	1·3	*Venus ovata*	3*j*	0·004
Glycera sp.	1		*Venerupis rhomboides*	1	0·14
Lumbriconereis sp.	6		*Cultellus pellucidus*	3	0·062
Cirratulidae	2		*Cellaria* sp.	+	0·001
?*Owenia* sp.	(6)		*Alcyonidium* sp.	1*j*	0·057
Pallasia murata	1		*Amphiura filiformis*	1	0·008
Amphicteis gunneri	1		*Cucumaria elongata*	1	0·11
Polychaeta indet.	(20)		*Labidoplax digitata*	1	0·48
Ampelisca spinipes	2	0·009			
Maera othonis	1		Total dry weight		2·5

Table XIX. Station C3. Numbers and Weights per $\frac{1}{2}$ m.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Anemone	1	1·1	*Processa* sp.	1	0·13
Nephthys sp.	1	0·68	Burrowing prawn*	1	
?*Owenia* sp.	(4)		*Dosinia lupinus*	1	0·043
Polychaeta indet.	15		*Tellina donacina*	1	0·032
Ampelisca spinipes	1	0·004	*Ophiothrix fragilis*	15	2·4
Maera othonis	1		*Polycarpa pomaria*	1	0·064
Ebalia cranchi	2	0·088	Total dry weight		4·5

* Damaged specimen of *Upogebia* or *Callianassa*.

The fauna corresponds to that described by Ford (1923) as the EcVg (*b*) community, i.e. a community characterized by *Echinocardium cordatum*, *Venus striatula* (=*gallina*) and *Abra prismatica*.

The fauna taken at each station is shown in Tables XX–XXII. No one species occurs in any great numbers, but even comparatively sparse species occur regularly at most or all of the stations.

The commoner species were:

Edwardsia sp.
Nephthys sp.
Glycera sp.
Lumbriconereis sp.
Magelona papillicornis
M. cincta
Pectinaria koreni
Chaetopterus variopedatus
Phyllochaetopterus sp.
Ampelisca tenuicornis
Nucula turgida
Thyasira flexuosa
Phacoides borealis
Cardium echinatum
Dosinia lupinus
Abra prismatica
Cultellus pellucidus
Cellaria sp.
Amphiura filiformis
Ophiura affinis
Echinocardium cordatum
Labidoplax digitata

Venus striatula, although a 'characteristic' species in the community described by Ford, occurs only at two out of the nine stations. Its distribution is sufficiently restricted to set the stamp on the community which it inhabits, and its continued use as a characteristic species therefore seems justified. The writer has nowhere in the Plymouth area found it in any abundance.

The occurrence of *Glossobalanus sarniensis* at one station is of interest—it has also been taken at B5 (Table VI), and has been subsequently taken in a new dredge (see Forster, 1953) by Mr G. R. Forster. In the *Plymouth Marine Fauna* (Marine Biological Association, 1931) the only Enteropneusta recorded are tornaria larvae of *Balanoglossus*, which are sometimes taken in plankton-nettings at Plymouth. The tornaria larvae are presumably larvae of *Glossobalanus* from these grounds. The adult was not taken in the survey of Allen (1899), probably because the dredges used did not dig sufficiently deeply. The same species has also been taken at Salcombe by Dr D. P. Wilson (see Burdon-Jones, 1953).

Details of the stations are given below:

Station A6

50° 9′ N., 4° 15′ W. Depth: 64 m. Clean sand. 21. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

Station A7

50° 7′ N., 4′ 15′ W. Depth: 70 m. Clean sand. 21. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

Station B4

50° 12′ N., 4° 10′ W. Depth: 55 m. Slightly muddy fine sand. 18. v. 50. Area: 0·5 m.^{2}. Sieve: 1·2 mm.

TABLE XX. NUMBER AND WEIGHTS PER $\frac{1}{2}$ M.^{2}

(For further details see Tables X and XI.)

Station	A6		A7		B4	
	No.	Weight	No.	Weight	No.	Weight
Hydroid	+	< 0·001	+	0·19	+	0·36
Edwardsia sp.	10*j*	0·19	1*j*	0·024	1*j*	0·014
Anemone indet.	..	..	1	0·12	1	0·18
Porifera	..	..	..	..	1*j*	< 0·001
Nemertinea	1	0·42	1	1·3	3	1·3
Aphroditidae	..		..		3	
Oxydromus sp.	..		..		2	
Hesionidae	..		..		1	
Nereidae	..		1		..	
Nephthys sp.	1		4		(11)	
Glycera sp.	..		1		2	
Lumbriconereis sp.	5		4		16	
Goniada sp.	..		..		1	
?*Staurocephalus rudolphi*	..		..		1	
Scoloplos armiger	..		..		2	
Magelona cincta	3		..		3	
Cirratulidae	..		..		9	
Capitellidae	..		1		16	
Maldanidae	1		(3)		(6)	
Pectinaria koreni	..		2*j*		..	
Ampharetidae	..		..		1	
Sabellidae	1		1		..	
Polychaeta indet.	(4)		(6)		20	
Chaetopterus variopedatus	..	..	1	*0·50*	3*	..
Phyllochaetopterus tubes	1	..	2	..	1	..
Diastylis laevis	..	..	..	..	2	0·001
Ampelisca tenuicornis	..	..	1	0·001	3	
Bathyporeia tenuipes	..	..	..		1	
Pseudoprotella phasma	..	..	1			
Galathea sp.	..	..	1	0·25	..	..
Ebalia cranchi	..	..	..	..	1	0·010
Eulima sp.	..	..	..	..	1	0·001
Nucula turgida	1, 2*j*	0·032	1	0·025	..	..
Nucula nucleus	..	..	..	..	2, 5*j*	0·050
Thyasira flexuosa	3	0·018	7	0·065	..	..
Phacoides borealis	1	0·061	1	0·086	2, 1*j*	0·11
?*Montacuta ferruginosa*	..	..	..	..	1	0·001
?*Cardium echinatum*	1*j*	0·001	1*j*	0·003	..	..
Dosinia lupinus	1*j*	0·001	1*j*	0·006	3, 2*j*	0·45
Venus striatula	1, 1*j*	0·051	..	..	..	..
Abra alba	..	..	..	..	1, 1*j*	0·004
Abra prismatica	1*j*	0·001	1	0·022	1	0·011
Solecurtus chamasolen	..	..	..	..	2	1·9
Cultellus pellucidus	7, 13*j*	0·30	1, 8*j*	0·058	2, 4*j*	0·024
Hiatella arctica	..	..	2	0·042	..	..
Cellaria sp.	..	..	+	0·002	+	0·91
Polyzoa indet.	..	..	..	..	+	0·036
Amphiura filiformis	2	0·012	..	..	1	0·001
Ophiura affinis	3	0·005	3	0·003	..	..
Echinocyamus pusillus	..	..	..	..	4	0·002
Echinocardium cordatum	1	*0·21*	2, 2*j*	0·41	3	1·1
Labidoplax digitata	1	*0·48*	..	..	1	*0·48*
Leptosynapta inhaerens	..	..	1	*0·48*	..	..
Glossobalanus sarniensis	1	*0·55*	..	..	..	..
Total dry weight		2·3		3·6		6·9

* Empty tubes.

TABLE XXI. NUMBERS AND WEIGHTS PER ½ M.^{2}

(For further details see Tables X and XI.)

Station	B5		B6		B7	
	No.	Weight	No.	Weight	No.	Weight
Hydroid	+	0·026	+	0·92	+	0·45
Edwardsia sp.	7*j*	0·097	9*j*	0·13	3*j*	0·042
Cerianthus lloydi	1	0·015	..	..	..	..
Anemone indet.	..	..	1*j*	0·007	..	..
Nemertinea	2	1·3	1	2·0	2	0·46
Sigalion mathildae	..		1		..	
Aphroditidae indet.	1		2		..	
Phyllodoce sp.	..		2*j*		..	
Nereidae	..		1		..	
Nephthys sp.	3		3		3	
Glycera sp.	1		..		1	
Eunice harassi	..		1		..	
Lumbriconereis sp.	11		8		2	
Magelona papillicornis	..		..		1	
Magelona cincta	3		2		..	
Capitellidae	5		1		..	
Maldanidae	(5)		2		1	
Owenia sp.	1*j*		..		..	
Pectinaria koreni	..		1		†1	
Terebellidae	..		3		..	
Polychaeta indet.	5		9		3	
Chaetopterus variopedatus	1, 1*j*	*1·2*	2	*2·3*	..	..
Phyllochaetopterus tubes	+	..	+	..	+	..
Scalpellum scalpellum	1*j*	0·006	1*j*	0·001	..	..
Ampelisca tenuicornis	5	0·002	1	<0·001	..	..
Galathea sp.	..	..	3*j*	0·018	..	..
Eupagurus sp.	1*j*	0·005	..	..	..	..
Eurynome aspera	..	..	1*j*	0·14	..	..
Nucula nucleus	1	0·003	2	0·028	..	..
Nucula turgida	3	0·008	2	0·010	1	0·003
Chlamys sp.	..	..	..	..	3*j*	0·004
Thyasira flexuosa	..	..	2	0·018	3	0·022
Phacoides borealis	3, 1*j*	0·16	..	..	3	0·18
Montacuta ferruginosa	..	..	..	..	1	0·004
Cyprina islandica	1*j*	0·003	..	..	1	7·6
Cardium echinatum	1	3·6	..	..	..	..
?*Cardium echinatum**	4*j*	0·018	..	..	3*j*	0·008
Dosinia lupinus	1	0·094	1	0·42	1	0·46
Tellina donacina	..	..	1*j*	0·003	..	..
?*Abra prismatica*	..	..	2	0·022	..	..
Cultellus pellucidus	1, 7*j*	0·025	5, 7*j*	0·16	5	0·019
Hiatella arctica	..	..	..	..	1*j*	0·003
Cochlodesma praetenue	..	..	1	0·017	1*j*	0·003
Cellaria sp.	+	0·012	+	0·037	..	..
Polyzoa indet.	+	0·024	+	0·025	..	..
Ophiothrix fragilis	1	0·003	2	0·24	..	..
Amphiura filiformis	3	0·004	..	..	..	..
Ophiura affinis	1	0·002	1	0·002	3	0·002
Echinocyamus pusillus	..	..	..	..	1	0·001
Echinocardium cordatum	2	*0·55*	..	..	2	*0·55*
Cucumaria lactea	..	..	5	0·070	..	..
Labidoplax digitata	2	*0·97*	1	*0·48*	2	*0·97*
Total dry weight		8·1		7·0		10·8

* Small specimens, which could not be identified with certainty.
† Identity doubtful.

TABLE XXII. NUMBERS AND WEIGHTS PER $\frac{1}{2}$ M.^{2}

(For further details see Tables X and XI.)

Station	C4		C5		C6	
	No.	Weight	No.	Weight	No.	Weight
Hydroid	..	..	+	0·040	+	0·66
Edwardsia sp.	1*j*	0·014	1*j*	0·019	3*j*	0·042
Cerianthus lloydi	..	..	..		1	0·23
Anemone indet.	1	0·36	2		..	..
Nemertinea	..	..	3	1·7	2	2·0
Sthenelais limicola	1	1·2	..		..	
Aphroditidae	2		..		3	
Nereis sp.	..		..		3*j*	
Nephthys sp.	2		2		5	
Glycera sp.	3		5		2	
Lumbriconereis sp.	3		9		18	
Nerine sp.	..		..		1	
Magelona papillicornis	..		2		2	
Magelona cincta	1		5		12	
Maldanidae	..		..		1	
?*Owenia* sp.	..		..		1	
Pectinaria koreni	2*j*		?3*j*		?7*j*	
Polychaeta indet.	3		5		13	
Chaetopterus variopedatus	..	..	..	..	2	*2·3*
Phyllochaetopterus tubes	..	..	..	..	+	..
Scalpellum scalpellum	..	..	..	..	5	0·083
Leucothoë sp.*	..	..	..	..	1	0·001
Aorid	..	..	..	..	1	
Pseudoprotella phasma	..	..	..	..	1	
?*Porcellana longicornis*	..	..	..	..	1	0·015
Corystes cassivelaunus	1	1·5	1*j*	0·036	1*j*	0·042
Cylichna sp.	1	0·006	..	..	..	..
Nucula turgida	4	0·047	6	0·024	4	0·051
Thyasira flexuosa	8	0·043	9	0·050	5	0·044
Phacoides borealis	..	..	?1*j*	0·010	1	0·025
Cardium echinatum	..	..	1	4·0	..	..
?*Cardium echinatum*†	..	..	1*j*	0·010	..	..
Dosinia lupinus	1*j*	0·013	1	0·083	2*j*	0·017
Venus striatula	1*j*	0·001	..	..	..	..
Abra alba	1	0·011	1	0·003	1	0·050
Abra prismatica	1*j*		..	..	1, 3*j*	0·032
Gari fervensis	..	..	..	..	3	1·36
Solecurtus chamasolen	1	0·47	..	..	..	..
Cultellus pellucidus	14	0·11	14	0·11	23	0·096
Ensis ensis	1	0·73	..	..	..	..
?*Thracia pubescens*	..	..	..	..	1*j*	0·014
Cellaria sp.	+	0·001	+	0·010	+	0·010
Ophiothrix fragilis	..	..	..	..	1	0·051
Acrocnida brachiata	..	..	2	0·021	1	0·018
Ophiura affinis	2	0·001	3	0·002	1	0·001
Echinocyamus pusillus	..	..	..	..	2	<0·001
Echinocardium cordatum	4, 3*j*	*1·2*	2	*0·57*	3, 1*j*	*1·2*
Echinocardium sp.†	..	..	1*j*		4*j*	
Labidoplax digitata	..	..	1	*0·48*	1	*0·48*
Ascidiella aspersa	..	..	..	..	1*j*	0·007
Aphya pellucida‡	..	..	..	..	1	..
Total dry weight		5·7		7·2		8·8

* See p. 25.
† Small specimens, which could not be identified with certainty.
‡ The weight of this fish is not included.
A query (?) before the number of individuals indicates uncertain identity.

Station B5

50° 10′ N., 4° 10′ W. Depth: 60 m. Clean sand. 3. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

N.B. This is distinct from the series of hauls made at this station on 5. xii. 50, described on pp. 14–19.

Station B6

50° 8′ N., 4° 10′ W. Depth: 62 m. Clean sand. 3. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

Station B7

50° 6′ N., 4° 10′ W. Depth: 68 m. Clean sand. 3. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

Station C4

50° 11′ N., 4° 5′ W. Depth: 57 m. Clean sand. 27. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

N.B. This is distinct from the hauls made at this station on 7. xi. 50, described on pp. 21–22.

Station C5

50° 9′ N., 4° 5′ W. Depth: 62 m. 27. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

Station C6

50° 7′ N., 4° 5′ W. Depth: 66 m. 27. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

The total dry weight on these grounds ranges from 2·3 g. (A6) to 10·8 g. (B7) per $\frac{1}{2}$ m.^{2}. The mean figure for the nine stations is 6·7 g. The great variability in the catch from one station to the next is due to the occurrence of large lamellibranchs, *Cyprina* and *Cardium echinatum* in particular, which are present in some samples and not in others.

Dry weight at B5

The 0·5 m.^{2} sample of 3 July 1950, from a 2·2 mm. sieve, gave a figure of 8·1 g./$\frac{1}{2}$ m.^{2}. The 2 m.^{2} sample of 5 December 1950, from a 1·2 mm. sieve, gave 11·6 g./$\frac{1}{2}$ m.^{2}, as set out in Table XXIII. The difference is partly accounted for by the use of a different sieve, which resulted in a doubling of the weight of Polychaeta (other than *Chaetopterus*) taken with the finer meshed sieve; but for other groups the differences were not so great. Since the total weight was made up mostly of a few large individuals, the discrepancy is partly attributable also to sampling errors.

If we ignore the weights of the Polychaeta (other than *Chaetopterus*), and of large specimens of *Cardium echinatum* and *Cyprina islandica*, the totals become:

$\frac{1}{2}$ m.^{2} sample of 3. vii. 50: 3·2 g./$\frac{1}{2}$ m.^{2},
2 m.^{2} sample of 5. xii. 50: 3·3 g./$\frac{1}{2}$ m.^{2},

which is a reasonably good agreement.

TABLE XXIII. STATION B5, 5 DECEMBER 1950. DRY WEIGHTS PER 2 M.^{2}

For some species the weights obtained for each series of ten samples is also given (samples: 1–10/11–20/21–30/31–40.)

Assumed weights are in italics.

		Weight
Hydroid		0·42
Virgularia mirabilis		0·004
Cerianthus lloydi		0·047
Edwardsia sp.		0·25
Chaetopterus variopedatus	—/*1·1*/*1·1*/*1·1*	*3·3*
Other Polychaeta, Gephyrea	1·8/2·1/3·6/1·9	9·4
Amphipoda		*0·012*
Other Crustacea		0·37
Acteon tornatilis	0·001/0·001/—/—	0·002
Cylichna cylindracea	—/0·001/—/0·001	0·002
Nucula nucleus	0·001/—/—/—	0·001
Nucula turgida	0·013/0·006/0·008/0·037	0·064
Musculus marmoratus	—/0·001/—/—	0·001
Chlamys sp.*	—/0·006/—/0·11	0·116
Thyasira flexuosa	0·019/0·007/0·011/0·008	0·045
*Phacoides borealis**	0·39/0·087/0·26/0·26	0·997
Montacuta ferruginosa	0·007/—/0·006/—	0·013
Cyprina islandica	9·7/—/*9·7*/—	*19·4*
Cardium echinatum	0·015/—/—/4·2	4·215
Dosinia lupinus	0·94/0·047/0·49/0·19	1·667
Venus ovata	0·001/—/—/—	0·001
Venus striatula	0·004/0·11/0·52/—	0·634
Venerupis rhomboides	0·006/0·007/—/—	0·013
Abra alba	—/—/0·014/—	0·014
Abra prismatica	0·011/0·024/0·026/0·014	0·075
Gari fervensis	0·001/0·001/0·040/—	0·042
Cultellus pellucidus	0·080/0·043/0·097/0·11	0·330
Hiatella arctica	0·006/0·008/—/—	0·014
Cochlodesma praetenue	0·010/—/0·003/0·003	0·016
Lyonsia norwegica	—/—/0·003/—	0·003
Cellaria sp.		0·066
Ophiuroidea		0·13
Echinocyamus pusillus		$<$0·001
Echinocardium cordatum		1·2
Labidoplax digitata		*1·6*
Tunicata		0·63
Glossobalanus sarniensis		*1·1*
Total		46·2

* The following corrections are necessary when comparing with Table VI: *Phacoides borealis*, 8, *not* 9, specimens in samples 1–10; 8, *not* 10, in samples 21–30 (when the shells were opened for weighing three were found to be empty). *Chlamys* sp. A single specimen, not recorded in Table VI, was found in hauls 31–40.

SUMMARY OF BIOMASS DATA

For all stations, the mean dry weight of animal tissue is 11·2 g./m.^{2}, which is equivalent to about 55 g. fresh weight. While this figure is sufficient for comparative purposes, there are a number of corrections to be applied if an absolute figure is required. Apart from any errors due to the small size of the area sampled, the following sources of error are present:

(i) Animals missed by the instrument, either because they live too deeply in the deposit, or are able to evade the sampler. Since the magnitude of the error is unknown, no correction can be applied.

(ii) Loss of small animals through the sieve. It is believed that the use of a 1·0 or 1·2 mm. sieve, the usual mesh in such investigations, would increase the total dry weight taken by about 10% (p. 22).

(iii) Weight of sand in the guts, estimated as 5% of the alcohol weight (p. 24). Taking 27·72 as the mean percentage dry weight relative to the alcohol weight (p. 23), the corrected percentage becomes 22·72, i.e. the dry weight figure should be reduced by about 18%.

Applying corrections for sieving errors and gut content, a figure of about 10 g./m.^{2} dry weight is reached.

As already emphasized, much of the total dry weight is made up of large individuals, having a relatively low metabolic rate, and therefore of less importance from the aspect of productivity than their weight would suggest. The percentage of individuals, each weighing over 0·2 g. dry (= *c.* 1 g. fresh weight), is given below:

Station	Percentage over 0·2 g.	Station	Percentage over 0·2 g.
A1	0	B5	77·6
A2	76·9	B6	45·4
A3	22·2	B7	88·8
A4	58·5	C1	37·4
A5	90·1	C2	20·0
A6	53·2	C3	23·6
A7	46·0	C4	74·3
B1	100	C5	70·1
B2	72·6	C6	62·8
B3	60·2		
B4	50·0	Total	64·4

An analysis has been made of the percentages of the total weight made up by the different groups of the animal kingdom, both for all twenty stations and for the nine stations on the sandy grounds outside the Eddystone. These show the preponderance of Polychaeta, Lamellibranchia and Echinodermata; the only other groups occurring in significant quantities being Coelenterata and Malacostraca (the percentages are shown graphically in Fig. 7):

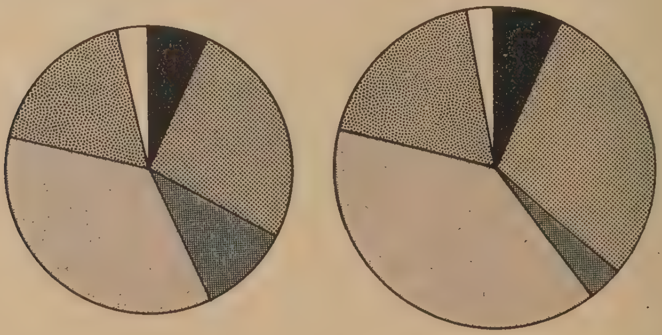

Fig. 7. Percentage composition by weight of the fauna. Left: all stations in the 1950 survey; right: stations on the Eddystone sandy grounds only. The area of the circles is proportional to the total biomass. ■, Coelenterata; ▒, Polychaeta etc.; ▓, Crustacea Malacostraca; ░, Lamellibranchia; ▨, Echinodermata; □, others.

	All stations	Eddystone sandy grounds
Coelenterata	7·00	6·84
Polychaeta and Nemertinea	25·79	29·49
Eumalacostraca	0·16	0·16
Malacostraca	10·35	3·38
Gastropoda	0·02	0·01
Lamellibranchia	35·46	39·10
Polyzoa	2·46	1·76
Echinodermata	17·88	18·33
Protochordata	0·88	0·93

The only major difference is in the low figure for Malacostraca on the Eddystone grounds, attributable to the scarcity of the burrowing prawns *Upogebia* and *Callianassa*.

CHANGES IN THE BOTTOM FAUNA

Allen's survey

Allen's (1899) survey of the bottom fauna in the neighbourhood of the Eddystone was largely based on dredge hauls. His grounds I–III and VI correspond with the Eddystone sandy grounds described in this paper. His species lists are mainly made up of members of the epi-fauna, among which hydroids figure prominently, but there is no reason to suppose that any major qualitative changes have occurred over the past fifty years.

One species, the scaphopod mollusc *Dentalium entalis*, is now conspicuous by its absence. Allen records this species alive from all fine sand grounds, with the exception of haul 104 on ground II, where the absence of this species was noteworthy. In the past few years no living *Dentalium* has been taken at Plymouth, although empty shells are very common. Specimens have recently been taken, however, in dredge and grab hauls in the mouth of the Channel, south of Land's End.

Ford's survey

The survey by Ford (1923) provides data on the density of the bottom fauna in 1922–23 for many positions off Plymouth. Any comparison with the 1950 results is complicated, however, by: (i) Ford used the $\frac{1}{10}$ m.^{2} Petersen grab, which has been shown (pp. 11–12) to sample less efficiently than the scoop-sampler used in the 1950 survey. (ii) The patchy nature of the inshore grounds and the fact that the same positions were not revisited in 1950 makes any exact comparison impossible. Ford did not sample the sandy grounds outside the Eddystone. (iii) Any comparison can only be in terms of numbers of animals, irrespective of their size or weight, since Ford gives no such data. (iv) Ford used the finest sieve in the *Challenger* series, which has openings of about 1·2 mm., whereas a 2·2 mm. sieve was used in 1950. (v) The samples described by Ford were selected to illustrate the different types of community to be found, and some of the less productive samples were omitted. For the same reason stations tend to be aggregated in the richer areas.

Mr Ford has kindly lent me his original notes, which include records of samples not published in his paper; all his stations within the area covered in 1950 are used in the comparisons which follow. To eliminate any bias due to aggregation of Ford's stations, those from certain areas have been averaged and scaled down so as to contribute only ½ m.^{2} to the total. The samples, positions of which are shown in Fig. 1 (p. 4), are summated thus.

	Sampled (m.^{2})	Reduced to (m.^{2})
Whitsand Bay	4·8	0·5
Rame mud	3·7	0·5
Eddystone	3·1	0·5
Mewstone	2·1	0·5
Other samples	6·2	6·2
Total		8·2

For comparison, the following 1950 stations have been taken: A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, C1, C2, C3, C4. The total area is 7 m.^{2}, and figures for both surveys have been converted to densities per 10 m.^{2}. (Two small divergencies in the 1950 survey have been ignored—these are the use of a 1·2 mm. sieve at B3 and B4 and the sampling of 0·6 m.^{2} instead of 0·5 m.^{2} at A3.)

A comparison of the lamellibranch faunas is given in Table XXIV. Before considering this in detail, allowance must be made for the different sieves used. The 2·2 mm. sieve used in 1950 had square apertures of *c.* 3 mm. diagonal, so that a shell having a breadth of less than 3 mm. may pass through end-on.

It is difficult to estimate the percentage losses due to use of the coarser sieve. Station B 5 was sampled on two occasions: on the first (Table XXI) an area of 0·5 m.^{2} was sieved through the 2·2 mm. sieve, on the second (Table VI) an area of 2 m.^{2} was sieved through a 1·2 mm. mesh. The results indicate that the coarser sieve retained over half (57·5 %) of the numbers retained by the 1·2 mm. sieve. The figure for the razor-shell, *Cultellus*, is 80 %.

At C 4 (Table VIII) the sample of 7 November 1950 was passed through first the 2·2 mm. and then the 1·2 mm. sieve. Only 32 % of the total was found on the coarser sieve.

If the samples from A 1, where a 2·2 mm. mesh was used, and from the nearby station in Whitsand Bay (1·2 mm. mesh used) are compared, a figure of 51 % is obtained as the percentage retained by the coarser sieve.

It is not claimed that any of the above comparisons have any great validity, but if we take 50% as the correction to be made, no great error should result.

Ford (1925) gives some data on the growth of lamellibranchs, from which it follows that spat of the larger species very soon grow sufficiently large to be retained by a 2·2 mm. mesh. *Abra alba*, for which species most data is available, has a shell breadth equal to c. 72% of its length. Individuals up to about 4 mm. would therefore pass a sieve of this mesh. Of a total of 7391 individuals measured from samples taken through the year in Bigbury Bay (east of Plymouth), only 9·16% were in the 4 mm. length group or below. The percentage varied from nil to 90% according to the season.

Spisula elliptica spat grew from 3·44 to 6·18 mm. length in 20 days in July and *Lutraria lutraria* increased from 3 to 12 mm. in 6 weeks. Individuals of *Cultellus* spatted in June 1922, grew to 15–20 mm. in length by the autumn of the same year. Since the shell breadth of *Cultellus* is some 20% of its length, it follows that individuals up to 15 mm. length might pass the sieve. But an analysis of the lengths of shells taken in the 2·2 mm. sieve in 1950 shows that many below this length were retained. Of 116 specimens taken 29 (25%) were 15 mm. or over, 74 (63·8%) were between 10 and 15 mm., and 13 (11·2%) were less than 10 mm. There is no reason to suppose, therefore, that losses of *Cultellus* were exceptionally high.

Where small species such as *Nucula* and *Corbula* are concerned losses through the coarse sieve may be considerable.

The sampling capabilities of the Petersen grab and the scoop-sampler vary with the nature of the sediment, and therefore the relative degree of penetration by the two samplers and also with the depth of individuals in the sediment. On the whole, older individuals (particularly of lamellibranchs) tend to be deeper down in the sediment. Data given in Table III, also in Holme (1949), suggest that the Petersen grab takes half or less than half as many individuals per unit area as does the scoop-sampler.

It therefore follows that two corrections must be applied to the data in Table II. The 1950 results must be doubled to allow for sieving error and the 1922–23 results doubled to allow for individuals missed by the Petersen grab. The *ratios* of the two sets of sample can therefore be taken direct from the table.

Of the differences in total population numbers shown in Table XXIV, the greater part is due to two species, *Abra alba* and *Cultellus*.

Taking the number of individuals of *Abra* which would have been lost had the coarse sieve been used as 9%, the population decline over the period is seen to be to 3·6% of its original value. There is reason to believe that *Abra* was exceptionally abundant at the time of Ford's survey; indeed Ford (1925, pp. 545–7) predicted that the dense bed in Bigbury Bay would soon become relatively barren owing to lack of replacement stock. The writer has been unable to locate any dense beds of *Abra* during the last few years.

The figures for *Cultellus* show a decline from 188·1 to 50·0. Sieving losses have already been shown not to be as high as might be imagined, and it seems fairly certain that a decline in population density, of the order of 75%, had occurred. The apparent decline in the population of *Nucula turgida* may be due to sieving losses in the 1950 survey (see above).

The differences in numbers of other species shown in Table XXIV cannot be considered significant, although they may indicate trends which can be confirmed at a later date. The totals for all species except *Cultellus* and

Table XXIV. Comparison of Lamellibranch Faunas in 1922–23 and 1950. Numbers per 10 m.^{2}

(The identity of the *Lutraria* taken in 1950 is uncertain. Some other doubtful identifications are included in the 1950 results, e.g. small specimens of ? *Cardium echinatum*)

	1922–23	1950
Nucula nucleus	23·2	11·4
Nucula hanleyi	1·9	..
Nucula turgida	56·6	11·4
Glycimeris glycimeris	13·9	..
Modiolus phaseolinus	..	1·4
Musculus marmoratus	1·2	..
Chlamys opercularis	0·3	4·3
Lima loscombi	0·8	..
Thyasira flexuosa	4·2	11·4
Myrtea spinifera	4·2	..
Phacoides borealis	..	11·4
Diplodonta rotundata	1·2	..
Lepton squamosum	..	2·8
Montacuta ferruginosa	0·3	1·4
Mysella bidentata	1·2	..
Cyprina islandica	1·2	1·4
Cardium echinatum	14·8	8·6
Cardium ovale	0·5	..
Cardium scabrum	0·4	1·4
Cardium crassum	0·5	..
Dosinia lupinus	4·9	17·1
Gafrarium minimum	11·8	..
Callista chione	0·8	..
Venus casina	1·2	..
Venus ovata	14·6	4·3
Venus fasciata	16·8	1·4
Venus striatula	12·1	1·4
Venerupis rhomboides	6·7	1·4
Mysia undata	..	1·4
Donax vittatus	2·2	..
Tellina pygmaea	2·6	..
Tellina fabula	..	7·1
Tellina donacina	..	1·4
Tellina crassa	2·8	8·6
Abra alba	107·8	7·1
Abra nitida	0·7	..
Abra prismatica	37·8	2·8
Gari fervensis	1·2	..
Gari tellinella	4·8	1·4
Solecurtus scopula	0·2	1·4
Solecurtus chamasolen	1·5	25·7
Cultellus pellucidus	188·1	50·0
Ensis ensis	5·5	2·8
Ensis arcuatus	..	1·4
Mactra corallina	3·7	1·4
Spisula elliptica	5·7	..
Spisula subtruncata	0·5	..
Lutraria lutraria	9·9	1·4
Corbula gibba	15·2	..
Thracia villosiuscula	0·4	..
Thracia convexa	0·2	..
Total	576·1	206·4

Abra alba are 280 for 1922–23 and 149 for 1950. The decline in population numbers appears therefore to be of the order of 50%.

Figures for the brittle-star *Ophiothrix fragilis* show an increase in numbers over the period. The species usually occurs in dense and localized beds (Vevers, 1951) that this difference may well be fortuitous. Most of the *Ophiothrix* obtained in 1950 were in one or two individual hauls at A5 and C3.

The population of the heart-urchin *Echinocardium cordatum* was 150 in 1922–23 and 18 in 1950. This species must quickly reach a size which does not pass the sieves, so that the decline in numbers is probably greater than the figures would indicate. Many young individuals were taken in the earlier survey.

While there can be little doubt that there has been a decline in the *numbers* of certain invertebrates, it is not possible to indicate what changes have occurred in the biomass, since no information is available on the exact sizes of individuals taken in Ford's survey.

Mare's survey

Mare (1942) gives a figure of 75 g./m.^{2} fresh weight for the larger macrobenthos in the Rame mud in 1939. At A2, a mile or two to the south, there was *c.* 50 g./m.^{2} in 1950, and the average for all 1950 stations was *c.* 55 g. (p. 37). It is possible that there had been a slight decline since 1939, but the differences might also be due to sampling errors.

DISCUSSION

The biomass figures given in this paper are a measure of the standing-crop of the macrofauna on the sea-bed off Plymouth during 1950. They are intended to provide basic data for following changes in the bottom-fauna in the future.

Before any attempt can be made to correlate the density of the fauna with long-term hydrographic or other changes it will be necessary to know the extent to which the populations fluctuate under constant conditions. Few data, apart from that given by Ford (1925) for some lamellibranchs are available for the Plymouth area.

It is well known that marine populations fluctuate from time to time. Petersen (1918), for example, gives figures showing changes in the bottom-fauna of two Danish fjords over a number of years. While seasonal changes do occur, particularly in shallow water, these are far outweighed by the year to year changes.

Ursin (1952) records considerable changes in the fauna of the Dogger Bank since the survey by Davis (1923). Between 1922 and 1951 numbers of *Spisula subtruncata* and *Mactra corallina* showed a marked decline, while other species had increased in numbers. Birkett (1953), however, considers that these changes may be partly due to seasonal variations in populations and to differences in sampling gear.

Major fluctuations appear to be due to the success or otherwise of spat-fall in each year. It follows that these fluctuations are mainly influenced by the suitability of conditions for the growth and development of pelagic larvae. Thorson (1950) considers that survival during larval life is affected mainly by the availability of suitable food and also by the effects of predators. Species with a long larval life tended to show greater fluctuations in their adult populations than species with a shorter larval life. This is due to the greater risks resulting from a long pelagic life which might in some years result in scarcely any larvae reaching the settling stage. Thorson (1950, p. 11) states that over 70% of marine invertebrates have pelagic larvae with a relatively long larval life. He believes that the mortality at the settlement stage is not as great as might be thought since it is now known that many species can postpone settlement until they drift over a suitable substratum (Wilson, 1952).

Many instances could be quoted where population fluctuations appear to be associated with good and bad settlement years. Among these are Ford (1925) and Stephen (1931, 1932) for lamellibranch populations.

Great variations may occur in the density of species where soils of varying grade occur within the same area. In the stations worked in 1950 the dry weight ranged from 2·6 g. (St. C1) to 21·4 g. (St. B7)/m.^{2}. Jones (1951, p. 139) records even greater variations of weight off the Isle of Man. The biomass is not necessarily related to the quantity of organic matter in a deposit, but seems rather to be related to the suitability of the deposit as a habitat for particular species. For example shell-gravel of a particular grade, as at A5, is favourable for the development of a rich fauna of lamellibranchs and *Amphioxus*, but if the shell gravel is of a slightly different grade it no longer provides a suitable habitat and is relatively barren.

Davis (1923) and Orton (1937) have emphasized the importance of currents and eddies respectively in the dispersal and settlement of larvae. These may result in the patchy settlement of larvae over a relatively uniform deposit.

The fact that very high population densities are found in certain localized areas suggests that the space requirements of individuals do not normally limit density. Thus Davis (1923) records up to 8250 small *Spisula subtruncata*/m.^{2} on the Dogger Bank, and Stephen (1928) records populations of *Tellina tenuis* on the shore of up to 7588/m.^{2}.

Although benthic populations vary greatly in time and place, their density within an area must clearly bear some relation to the fertility of the overlying water mass. The food of most species during pelagic larval life and of suspension feeders in the adult population is the plankton, and since fluctuations in phyto- and zoo-plankton are closely linked with the supply of nutrient salts, it follows that the density of benthic species must similarly be affected.

Raymont (1947, 1949, 1950) has shown that the addition of fertilizers to an enclosed sea-loch and to an arm of an open sea-loch resulted in a marked increase in the overall density of the bottom fauna. In open sea areas no comparable data are available, but it should be appreciated that changes in the fertility of the water do not necessarily affect all species in the same way. Thus an increase in fertility results not only in a greater supply of food for both larvae and adults but also in an increase in the number of predators. Raymont's results suggest, however, that an increase in bottom population will result in spite of the larger number of predators.

At Plymouth there has been a decline in the fertility of the area since about 1931, associated with the replacement of water characterized by *Sagitta elegans* by water characterized by *S. setosa*. This decline is measurable in terms of diminished phosphate and other nutrients in the water and by a decline in the number of young fish and of other organisms in the plankton. (The changes are summarized in Kemp, 1938, and Russell, 1939). The Plymouth herring fishery has died out, and Cooper (1948) has shown a decline in landings of the spur-dog (*Squalus acanthias*) associated with the fall in phosphate content of the water. In the present paper evidence has been given (pp. 39–43) of a probable decline in the numbers of lamellibranchs on the sea-bed since 1922–23.

Wilson (1951) has recently shown that other factors besides a decrease in the nutrient salt content of the water may be responsible for a decline in the density of the fauna. He has shown that water from off Plymouth is less favourable for the development of the larvae of certain benthic invertebrates compared with water taken to the westward in the Celtic Sea, which is characterized by *Sagitta elegans*.

It is natural that changes in the bottom-fauna should take place more slowly than in the plankton owing to the much longer generation time of the larger benthic invertebrates. Raymont (1947, 1949, 1950) found a delay of 2 or 3 years after the commencement of fertilization before anything like a maximal development of bottom fauna was obtained, and a similar lag of a year or so in the decline of the population after addition of fertilisers had ceased.

Many benthic species, particularly lamellibranchs, are believed to have a life span of many years, and the effects of a decline in fertility of the water might only become fully operative after a number of years when the adult spawning stock began to decline in numbers. Young of the lamellibranch *Cyprina islandica* appeared to be rare or absent in 1950 on grounds where the adult was quite common. While it is true that for a long-lived species the annual rate of replacement need only be small, is it possible that the 1950 population of adult *Cyprina* was spatted under more favourable conditions 20 years or more previously?

Apart from changes in the nature of the water masses, there is some evidence of a northward spread of warmer water species due to a small increase in temperature over the last half century. Spooner (1950) records certain species of amphipod which appear to have spread northward into the Plymouth area in recent years.

The uniformity of the fauna of the sandy grounds outside the Eddystone suggests that this would be the most favourable area for the study of long-term fluctuations in the benthic population. The fauna can be sampled more accurately than on the patchy grounds inside the Eddystone. The large number of species on this ground, none of which occurs in any great abundance, should tend to lessen year to year changes in the overall population density due to fluctuations in spatfall of individual species.

The only dense beds of invertebrates in the area seem to be the *Ophiothrix* beds recorded by Vevers (1951), so it seems unlikely that pelagic larvae of other invertebrates occur in dense aggregations and so give rise to localized spat falls, as on the Dogger Bank (Davis, 1923). The absence of eddy currents on offshore grounds should minimize the chance of local settlements associated with swirls as suggested by Orton (1937). In this connexion it is interesting to note that dense beds of Mollusca found off the Devon coast have all been in inshore areas where eddy currents may occur—these are the *Abra alba* bed in Bigbury Bay (Ford, 1923, 1925), the *Spisula solida* bed in Start Bay (Ford, 1925; Holme, 1950*a*) and the *Turritella communis* bed in Teignmouth Bay (Holme, 1950*a*).

SUMMARY

A survey has been made of the biomass of the macrobenthos at twenty stations in the English Channel off Plymouth. The object was to provide a basis for following long-term fluctuations in the fauna.

A brief survey of physical conditions in the area is given, and a grade-analysis of the soil at each station has been made.

Core-samples show that the sediment is shallow in many places and rock has been taken at 36 cm. or less below the surface at a number of stations.

Samples totalling $\frac{1}{2}$ m.^{2} were taken at each station with a modified 'scoop-sampler', covering $\frac{1}{10}$ m.^{2}, which is briefly described.

Sources of error in sampling are considered in detail. Some species may evade the sampler and others live too deep in the sediment to be taken. A comparison against a Petersen grab and a new 'suction-corer' show that the scoop-sampler does take a reasonably quantitative sample.

The number of species taken in successive hauls, when plotted against the log of the area sampled, approaches a straight-line relationship similar to that obtained by Williams (1950).

A statistical analysis is made of a series of samples taken at one station, and the variance between the two samples in each haul and between successive hauls calculated. At this particular station the fauna in successive samples is shown to be fairly random.

A sieve of 2·2 mm. mesh was employed. Compared with a finer sieve losses in terms of *numbers* may be quite large, but the total weight taken is little affected.

The methods used in dry-weight determinations are described in detail.

The fauna and dry weight at each station are listed. On grounds inside the Eddystone both fauna and deposits are patchy, but at nine stations further offshore a fairly constant fauna was taken.

The mean dry weight for all stations is 11·2 g. or *c*. 55 g. fresh weight/m.^{2}.

Much of the total weight is made up of large individuals. 64·4% is composed of animals weighing more than 0·2 g. dry (=*c*. 1·0 g. fresh weight).

The composition of the fauna in terms of different groups of animals is given—the greatest weights are Lamellibranchia (35%), Polychaeta (26%) and Echinodermata (18%).

A brief comparison is made with Allen's (1899) dredge survey. The only change noticed has been in the disappearance of *Dentalium entalis* from the outside grounds.

A comparison with the grab survey by Ford (1923) seems to indicate an appreciable decline in *numbers* of lamellibranchs compared with the 1922–23 figure. A considerable decline has occurred in populations of *Abra alba*, *Cultellus pellucidus* and *Echinocardium cordatum*.

The biomass figures obtained by Mare (1942) for the Rame mud do not indicate any considerable decline in the fauna between 1939 and 1950.

Fluctuations in benthic populations are briefly discussed, and it is emphasized that more data are required of seasonal and year to year fluctuations at Plymouth before any changes can be related to the fertility of the water-masses in the area.

The uniformity of the fauna on the outer grounds renders this area suitable for following changes in the bottom fauna.

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DEFAECATION IN RELATION TO THE SPONTANEOUS ACTIVITY CYCLES OF *ARENICOLA MARINA* L.

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(Text-figs. 1–6)

Four years ago, I showed that the behaviour of lugworms in sand could be studied by recording the water movements through their burrows, and that their activities are patterned in time, to a large extent, by inherent rhythms (Wells, 1949*a*, *b*). The following note describes a new series of experiments, in consequence of which the conclusions of the earlier papers are extended and modified in detail.

The work was done at Plymouth in July, August and September 1952. The expenses were in part defrayed by a grant from the Central Research Fund of the University of London.

METHODS

Three types of apparatus were used (Fig. 1).

Type I, suitable only for the smallest worms, consists of a glass U-tube (internal diameter 25 mm.) with arms of unequal length and a short side-tube (internal diameter 5 mm.) on the longer arm. Capillary C (internal diameter about 1 mm.) is connected by rubber tubing to the side-tube. The apparatus is immersed in a tank of sea water to the level L and partly filled with muddy sand (a mixture of clean sand with a black muddy clay from Millbrook) to the height shown by stippling in the drawing. Sea water flows continually into the tank and the level L is held constant by an overflow. If a healthy worm of suitable size is allowed to enter the sand, it makes a burrow with one end in each limb of the U-tube. Such water currents as it sets up must pass through capillary C, and, because of the slight resistance of the capillary, will cause changes of water-level in the long limb of the U-tube. These changes are recorded by the following means on a slowly moving kymograph (a Casella thermo-barograph clock turning once a day). A Palmer gimbal lever (amplification ×6) is connected by means of a thread and a blob of sealing-wax type adhesive to a circular cover-glass as used for microscope preparations (G). The cover-glass has a film of vaseline on the upper surface but is clean on the lower; it clings to the water surface, and is a great improvement on the paraffin floats used in my earlier work.

Types II and III differ from each other only in dimensions. Each consists of two rectangular glass plates held 22–24 mm. apart by a plasticene rim and centre-piece (cross-hatched in the drawing). The space between the plates is filled with muddy sand to a maximum depth of about 100 mm. in Type II and 300 mm. in Type III. The worms burrow in the sand, and their water currents are recorded by the system already described.

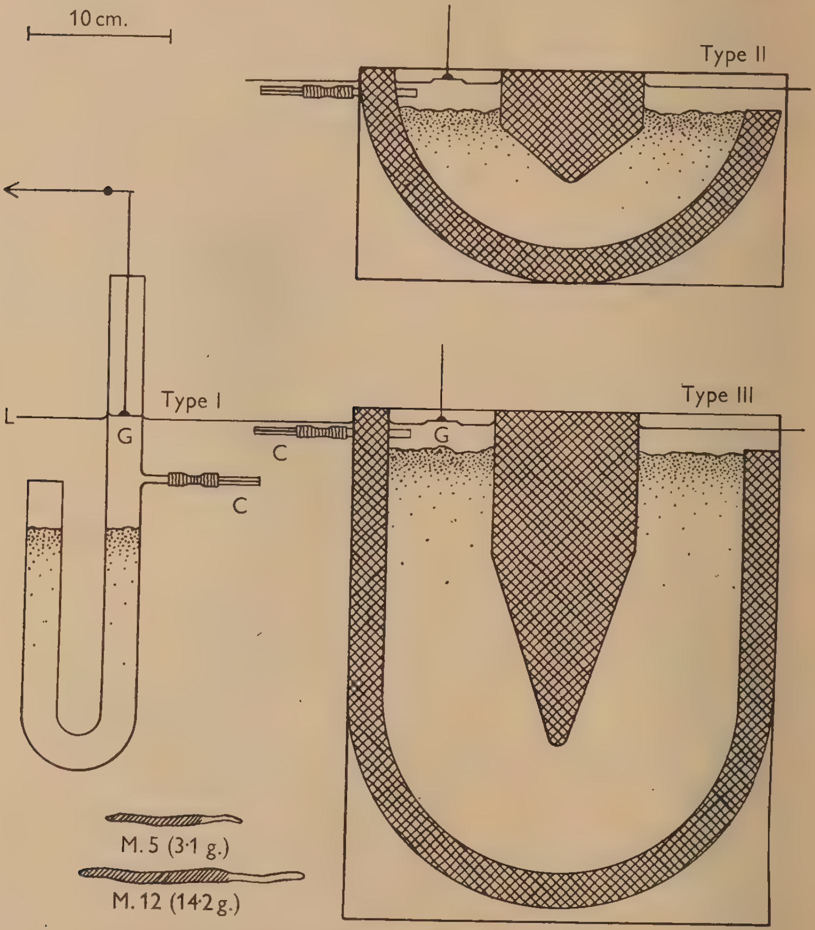

Fig. 1. Drawings of the three types of apparatus used. Outlines of two of the worms are included, to the same scale, in the lower left-hand corner.

The experiments were set up in groups of four. Fourteen individuals were used, the first two for 3 days only, while the apparatus was being tested and adjusted, and the remaining twelve for from 7 to 18 days. The temperature ranged from 16·1 to 17·9° C.

The worms are generally invisible in the sand, and defaecation is almost the only activity that can be directly observed.[1] The following steps were taken to correlate the timing of defaecation with the records of water movements. (i) Two or three times a day the experiments were examined, the presence or absence of faeces was noted, and any faeces seen were destroyed with a glass rod. (ii) On several occasions the experiments were kept under continuous observation for 4–6 hr., and the time of each defaecation was marked on the tracing and noted down to the nearest minute.

RESULTS

The worms often showed the characteristic behaviour pattern described in the following citations:

> Whenever the worms were feeding from a gradually subsiding cone and piling up castings, as they do in the field, a characteristic cyclical pattern was traced. This was marked by conspicuous diphasic excursions at intervals of about 40 min. Defaecation occurs at the summit of the first phase. By comparison with records got from worms in glass tubes, it is inferred that the first phase consists of tailward locomotion to the sand surface, and the second to headward irrigation accompanied by gentle creeping back to the feeding point. (Wells, 1949*b*, p. 477.)
>
> The pattern just described invariably accompanied feeding and defaecation. (Wells, 1949*b*, p. 471.)

These statements must be modified in two respects in the light of the new results. Firstly, the interval given as 'about 40 min.' varies considerably, one of the determining factors being the size of the worm; and secondly, defaecation may occur when the pattern is not discernible on the tracings.

Three examples of the typical pattern, taken from the new series of experiments, are seen in Fig. 2. The position of the base level (i.e. the point at which the lever settles when the capillary is removed) shows that the pattern is traced against a background of headward irrigation. At each of the conspicuous 'needles' on the tracings, the writing-point first moves towards the zero mark, as the worm ceases to irrigate and creeps backward to the surface of the sand. Then there is a very sudden further movement of the lever in the same direction (generally crossing the zero line) as a faecal cylinder shoots out. The act of expulsion takes about half a second, and the almost violent movement of the lever suggests that a powerful, piston-like swelling runs backward along the worm's body at this moment.[2] Immediately after defaecation, the lever moves towards its original position as the worm creeps down to the bottom of its burrow and begins to irrigate once more. The second phase of the diphasic cycle, consisting of unusually vigorous headward irrigation, follows at once; but the extent to which this second peak stands out from the general irrigation background is variable. Sometimes, as towards the end of Fig. 2C, it is barely discernible, and it may not emerge at all.

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[1] One occasionally sees the tail-tip appear at the caudal end of the burrow, move around a little as if clearing the opening, and then disappear again.

[2] Once during the earlier series of experiments I noted that the ejection of a cylinder was accompanied by a 'violent down-suck of water into the head shaft'. The fact that each 'needle' consists of two distinct parts is clearly shown in Fig. 3 of Wells 1949*b*.

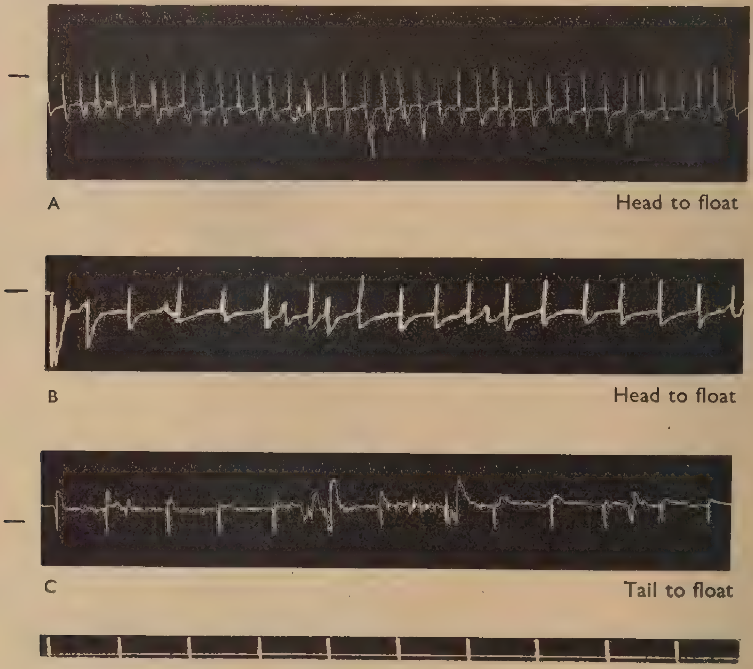

Fig. 2. Ten-hour records of frequently-defaecating worms.

A: worm M5 (3·1 g.). Apparatus Type I. Regular series of defaecations at average interval of 15 min. 20 sec.

B: worm M14 (10·1 g.). Apparatus Type III. Defaecations at average interval of 34 min. 20 sec.; occasional irregularities of the background irrigation between them.

C: worm M13 (12·1 g.). Apparatus Type III. Defaecations at intervals of about 45 min., with a rather irregular period in the middle of the record.

In all tracings: read from left to right; time signal marks once an hour; the mark to the left of each tracing is the zero-flow base level. The orientation of the worm relative to the float is printed under each extract. Wider capillaries were used for the larger worms, so a given lever excursion does not always correspond to the same flow rate.

The effect of size on the timing of the pattern is evident in Fig. 2. The experiments of 1948/9 were done on 'large' worms, unfortunately not weighed. The worms of the 1952 series ranged in weight from 2·6 to 18·6 g.

When defaecating regularly, the larger worms did so at intervals of 'about 40 min.', but the smaller gave much shorter intervals (15–20 min. for worms weighing about 3 g.).

Other types of record may be traced. In the 1948/9 experiments, the pattern of Fig. 2 appeared, accompanied by regular feeding and defaecation, during 45% of the total recorded time. The remaining records were grouped as follows: (i) cycles rather similar to those of Fig. 2 but without defaecation; (ii) periods of complete quiescence, which might last for many hours on end; (iii) periods of unexplained activity, the tracings of which were described as 'confused'. The records of the 1952 series include all of these types, and they also show a gradation of intermediate stages between the typical pattern of Fig. 2 and tracings which must be put in the 'unexplained' or 'confused' category. On several occasions, the worms defaecated frequently and quite regularly while 'confused' records were being written. The new results are reviewed in detail in the following paragraphs. Taken together, they suggest that the worms tend to settle into the pattern of Fig. 2 when conditions are favourable, and that limitation of space in the apparatus promotes 'confusion'.

Disregarding the preliminary tests, the 1952 material includes three sets of four worms each, those of the first set being the smallest and those of the third the largest.

Set I. Worms M3–6. (2·6–3·2 *g*)

These worms were studied in Type I apparatus. They burrowed vigorously and swiftly into the sand when placed on its surface, and their behaviour was recorded for 7 days. Extracts from the records are printed in Figs. 2A, 3 and 6C, D. The individual worms performed as follows.

M3 gave a few long spells of regular defaecation with needles like those of the first half of Fig. 3B—i.e. lacking a prominent second phase—and a few short spells (2–3 hr.) resembling Fig. 3A. Most of its tracings, however, were confused, with the defaecations few and irregularly scattered; there were several spells with pauses followed by defaecation, much as in Fig. 3C.

M4 was a consistently frequent defaecator. There were occasional short spells of irregularity, but most of its tracings were regular, like the examples in Figs. 3A and 6D. The second phase however, was not always distinct from the background irrigation.

M5 defaecated steadily for 5 days with only short spells of irregularity, its cycles being like those of Figs. 2A and 6C on some days but without a distinct second phase on others.[1] On the last 2 days the irregular periods increased in duration while the defaecation spells shortened correspondingly and finally ceased; and various confused patterns, including records resembling Fig. 3C, were traced.

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[1] On the 5th day, when extract 2A was taken, the worm moved nearly 10 c.c. of sand from one end of the tube to the other in 24 hr. As it defaecated about 100 times, this means a transport of 0·1 c.c. per defaecation. Weight of worm 3·1 g.; approximate length 9 cm. of which 2 cm. was tail.

M6 defaecated fairly frequently on most days, but gave confused records, including numerous spells of the type shown in Fig. 3C.

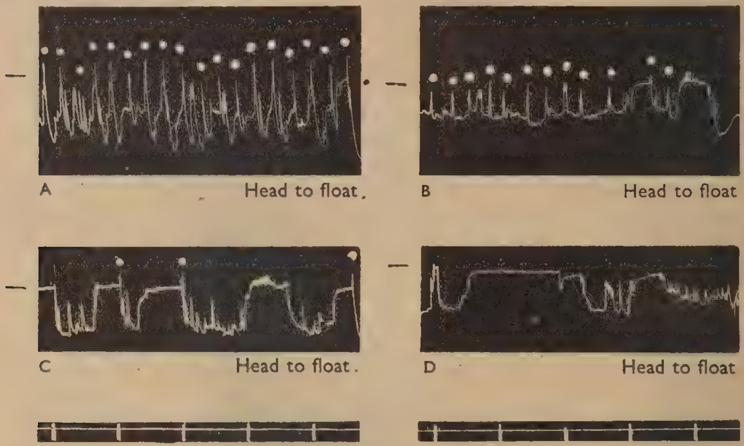

Fig. 3. Five-hour records traced by small worms in apparatus Type I. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M4 (2·6 g.). Intervals between defaecations 15, 16, 16, 15, 15, 16, 16, 18, 16, 17, 15, 18, 17, 18, 17, 18, 15 min. The record shows a pattern like that of Fig. 2A but against a more fluctuating background. There is a close correspondence between defaecation and the first peak of the diphasic excursion except that the 3rd defaecation is atypically placed; it occurs on a sharp needle sandwiched between two blunter ones.

B: worm M3 (3·0 g.). Intervals between defaecations 19, 15, 19, 14, 23, 17, 16, 16, 26, 38, 16 min. The defaecations occur on well-marked needles but there is no prominent second phase; the whole record becomes confused towards the end and defaecation ceases.

C: worm M6 (3·2 g.). Intervals between defaecations 58, 158 min. The record shows fluctuating activity alternating with periods of almost or quite complete quiescence; defaecation sometimes occurs as the worm 'wakes up' after a pause.

D: worm M3 (3·0 g.). A confused tracing with no defaecations.

Although these worms could burrow rapidly in the Type I apparatus, and often showed regularly spaced defaecations associated with typical diphasic excursions on the irrigation tracing, the results of Set II suggest that the rigid walls of the U-tubes may have been somewhat restrictive even for the small worms of Set I.

Set II. Worms M7–10 (7·0–8·0 *g.*)

This experiment lasted for a week. The worms were first put up in Type I apparatus, and transferred to Type II after 2 days (M7, 8) or 5 days (M9, 10).

When put into Type I, the worms began at once to make strenuous burrowing movements, but they had obvious difficulty in entering the sand.

This was doubtless because burrowing is done in the main by thrusting the sand aside, and only a small worm has room enough to do this in the U-tube. The worms of Set II could work their anterior segments into the sand, but they generally remained with the tail, or the tail and several branchiate segments, exposed at the surface. Only M10 was seen to get itself completely into the sand; this happened on two occasions, but each time its tail soon appeared again. Nevertheless, they all defaecated repeatedly and must therefore have fed. The tracings were of small amplitude, probably because part of the irrigation water returned along the limb of the U-tube where the worms were lying, and they were very confused in pattern.

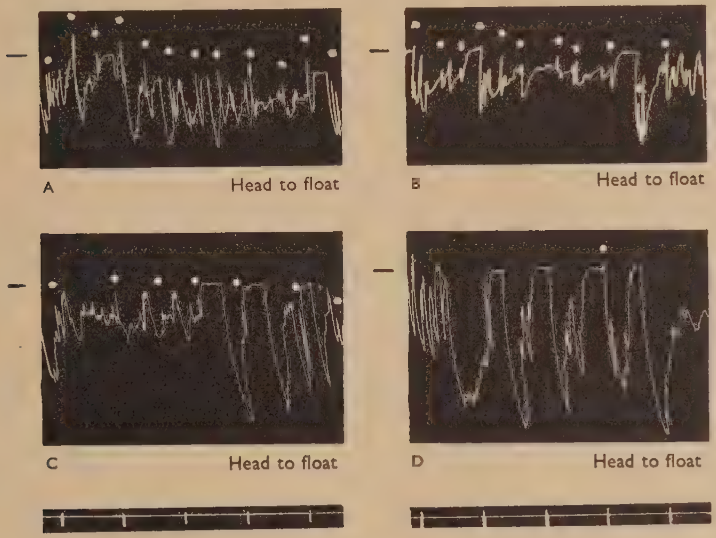

Fig. 4. Five-hour records traced by large worms in apparatus Type II. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M7 (7·3 g.). Intervals between defaecations 26, 23, 22, 23, 24, 25, 19, 30, 31, 24, 23 min. Base level drops suddenly about $1\frac{1}{2}$ hr. after start of extract, due to a change in rate of the sea-water supply to the tank in which the apparatus stood. Several of the defaecations are associated with diphasic excursions of the typical form, but many are not.

B: worm M14 (10·1 g.). Intervals between defaecations 29, 18, 19, 19, 19, 35, 17, 36, 27, 23 min. A confused tracing, the defaecation needles bearing no relation to the general irrigation pattern.

C: worm M12 (14·2 g.). Intervals between defaecations 58, 43, 37, 41, 56, 36 min. There is again no relation between the defaecation needles and the general irrigation pattern.

D: worm M7 (7·3 g.). A confused record, with one defaecation only. The pattern resembles that of Fig. 3C.

When transferred to Type II, the worms burrowed rapidly out of sight. Only M7 defaecated frequently under the new conditions, and its records were never of the simple cyclic pattern of Fig. 2. Extracts are given in Figs. 4A, D and 6B. The other worms defaecated rather seldom and gave confused tracings, e.g. Fig. 6A. Short spells of the curious rapidly-swinging rhythm seen between the reversals in Fig. 6A were shown by all these three worms, and the rhythm was once kept up quite regularly for about 6 hr. by M9. The worms of Set II were about 15 cm. long at full elongation, and the space available in the Type II apparatus was insufficient for the setting up of a burrow of the normal shape, even though they were able to descend rapidly into the sand.

*Set III. Worms M*11–14 (10·1–18·6 *g*.)

This experiment continued for 18 days, but M11 died on the 12th day. The worms were first put up in Type II apparatus, and transferred to Type III after 4 days (M11, 12) or 9 days (M13, 14). They burrowed rapidly down in both types.

The behaviour of the worms in Type II is illustrated by Fig. 4B, C. All of the worms defaecated frequently under these conditions, M13 being especially regular and consistent. Their tracings, however, are confused; there are occasional passages of 2–3 hr. during which the pattern of Fig. 2 is seen fairly clearly against an oscillating background, but generally, as in the extracts of Fig. 4, the defaecation needles have little or no relation to the rest of the irrigation tracing.

The behaviour in Type III is shown in Figs. 2B, C and 5. The case of M11 is exceptional. This worm became quiet after burrowing down in Type III and registered practically no water currents for 3 days; then it gave a rhythmic pattern without defaecation for about 12 hr. (Fig. 5C); then it suddenly became very active, tracing a graph like the usual recovery after a period of oxygen lack (as Wells, 1949*a*, fig. 6) followed by some hours of vigorous but confused activity; then its irrigation became less and less vigorous, until it died on the 12th day. The remaining worms often gave confused spells, sometimes with occasional defaecations. They also traced many long periods in which the defaecations came regularly and were associated with diphasic excursions of the usual kind (Fig. 2B, C), though this was sometimes written against a rather fluctuating background (Fig. 5B). A comparison of the records before and after the transfer leaves little room for doubt that the more spacious conditions of Type III favour the appearance of the regular pattern of Fig. 2.

Three rest periods, of 1–1½ days' duration, during which there was little or no irrigation, were shown by M12; these alternated with active periods. The pattern traced by M13 in Fig. 5A was not seen in any other record. It was presumably caused by a regular periodic reversal of the direction of travel of the irrigation waves along the worm's body.

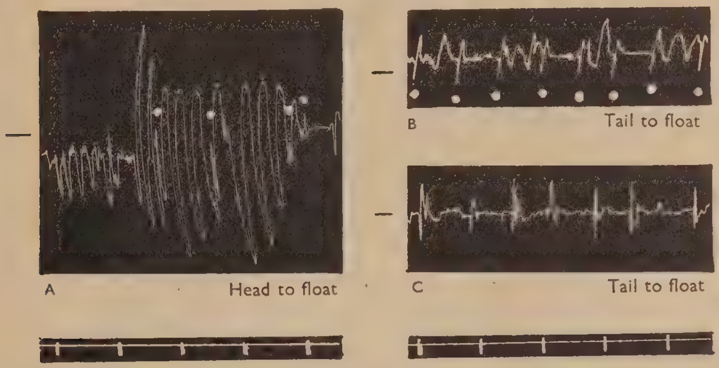

Fig. 5. Five-hour records traced by large worms in apparatus Type III. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M13 (12·8 g.). Intervals between defaecations 52, 74, 18 min. The record shows an unique irrigation pattern, with periodic reversal of the direction of flow.

B: worm M14 (10·1 g.). Intervals between defaecations 43, 39, 42, 36, 33, 37, 46 min. The defaecations occur on diphasic excursions of the typical form, against a fluctuating background.

C: worm M11 (18·6 g.). The record shows periodic outbursts of irrigation without defaecation.

Discussion

The relation between the irrigation patterns and defaecation may be summarized as follows.

Fasting worms in glass tubes often settle down, for many hours at a stretch, to a pattern consisting of periods of rest alternating with outbursts of activity (the *i–d* cycles, in the terminology of Wells & Albrecht, 1951). Each outburst typically consists of three phases—backward locomotion, headward irrigation with headward locomotion, tailward irrigation—but their form is variable in detail; the third phase, in particular, is often dropped out altogether. They sometimes appear with almost clockwork regularity, but their timing can vary, either after a period of oxygen lack or without obvious external cause. They apparently depend on a spontaneously discharging pacemaker whose activity can be controlled according to the circumstances, much as the beat of a vertebrate heart is spontaneous although its pattern and frequency can be regulated from without (Wells, 1949*a*; Wells & Albrecht, 1951).

Worms in sand, feeding and defaecating regularly, often show essentially similar cycles, except that they now appear against a continuous but often rather fluctuating background of headward irrigation (Fig. 2). Sometimes the worms in sand show a similar pattern when they are not passing food through the gut; and then, as in glass tubes, they generally rest between the outbursts (Figs. 5C, 6B).

Other spontaneous components may enter into the lugworm's behaviour. A second characteristic pattern of intermittent rhythmic activity originates in the oesophageal wall; it determines movements of the anterior end and proboscis accompanied by inhibition of the irrigation current (the *f* cycle of Wells & Albrecht, 1951). The irrigation records got from worms in sand sometimes show minor fluctuations between the *i–d* outbursts which may be due to the *f* cycle (Wells, 1949*b*, fig. 4; this paper, Fig. 2C). Additional types of periodic behaviour are occasionally indicated (Fig. 5A), but many of the tracings are confused, both in sand and in glass tubes, and it is often impossible to decide whether particular passages represent the appearance of something new, or modified derivatives of the *f* cycle (centre portion of Fig. 6A) or *i–d* cycle (Figs. 3C, 4D, beginning of Fig. 6A; compare Wells & Albrecht, 1951, Fig. 4).

Defaecation is frequently coupled with great regularity to the *i–d* cycles, with one defaecation on the first phase of each outburst (Fig. 2). In favourable circumstances, and given plenty of space, the worms tend to settle into this routine for many hours on end, and there is little doubt that they spend much of their time in this way in the field. However, as we have seen, it is not a necessary condition for the appearance of the *i–d* cycles that the worms should feed and defaecate; neither is it a necessary condition for defaecation that a regular irrigation pattern of this type should be traced.

The advantages of basing the organization in time of the lugworm's activities on spontaneous pacemaker systems, rather than on *ad hoc* reflexes to its various needs as they arise, were discussed elsewhere (Wells, 1949*a*, *b*). The *i–d* cycle is well suited to serve as a means of integration when the worm is living in an established burrow of the typical form. It brings the worm alternately to the place of feeding and the place of defaecation; it can be modified according to the composition of the water and the worm's respiratory needs; under low-tide conditions, it can be adapted for aerial respiration. Some method of coupling defaecation to the *i–d* cycle is therefore to be expected. On the other hand, the coupling should not be too rigid, for circumstances might arise in which the *i–d* pattern would be inappropriate. The worms of Set II fed and defaecated regularly in the Type I apparatus though unable to burrow wholly into the sand; this indicates a flexibility of behaviour which may sometimes be of service under natural conditions.

The mechanism of coupling appears to involve more than one factor. It is suggestive that defaecation often occurs as the worms 'wake up' suddenly and begin to irrigate in a headward direction after a period of rest (Figs. 3C, 4D). Similarly, in a typical *i–d* outburst, it is at the momentary pause at the top of the first phase, just before the onset of headward irrigation, that defaecation takes place (Fig. 2). The headward irrigation cannot be regarded as a conse-

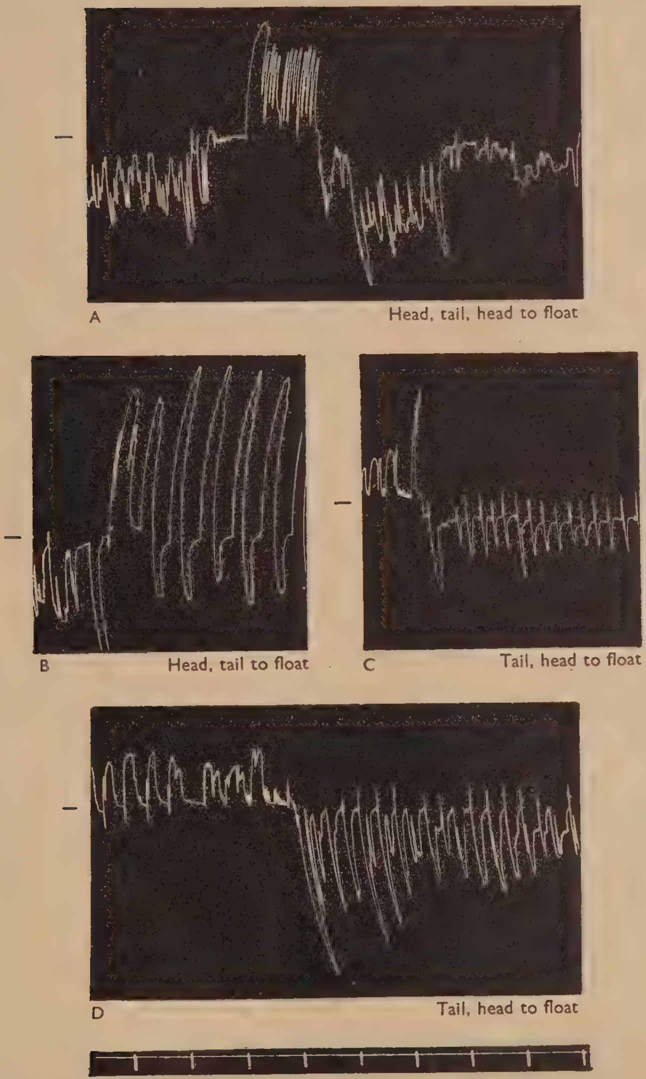

Fig. 6. Five- and nine-hour records to show reversal of the worms in their burrows.

A: worm M8 (8·0 g.). Apparatus Type II. A very confused tracing, with no defaecations. The worm reverses itself twice, with about 1½ hr. between reversals.

B: worm M7 (7·3 g.). Apparatus Type II. Reversal is followed by a regularly spaced series of irrigation outbursts without defaecation.

C: worm M5 (3·1 g.). Apparatus Type I. Reversal is followed by a regular series of defaecations with diphasic excursions of the usual form.

D: worm M4 (2·6 g.). Apparatus Type I. Defaecation occurs four times at the beginning, and frequently after reversal.

quence of the defaecation, because resting worms can 'wake up', and the *i–d* outburst can assume its typical form, in the absence of defaecation. Possibly, therefore, the onset of headward irrigation is preceded by some internal change which acts as a stimulus for defaecation, provided that other conditions (including a certain loading of faeces in the gut) are satisfied. But this is not the whole story, for the worms may defaecate at other times (Fig. 4B, C).

The role of rectal distension could perhaps be studied by collecting and weighing the individual faecal cylinders. Rough observations on the dimensions of the faeces were made during the present work, from which two points emerge. Firstly, in a regular series of defaecations (as in Fig. 2 or Fig. 3A), the faeces are always large and uniform in size. This suggests that the frequency of the *i–d* cycles can be adjusted to fit in with the rate of feeding, and the slight variations in the intervals between successive outbursts which appear even in the most regular series in sand may be due to this cause (see details in legends to Figs. 3–5). Secondly, when faeces are less regularly produced, their size may vary considerably. Thus, three of the cylinders produced during Fig. 3B (the first, tenth and eleventh) were conspicuously smaller than the rest, and these appeared after the longest intervals (27, 26 and 38 min. respectively). The last result is rather unexpected, and shows that factors other than rectal distension and a particular inflexion of the irrigation graph can influence the timing of defaecation.

To sum up, the following conclusions may be drawn. (i) Defaecation and the *i–d* outbursts are essentially independent, cyclically repeated events. (ii) When the worm is living under favourable conditions in an established burrow, the familiar pattern in which defaecation is closely coupled to the *i–d* cycle gives a workable means of integrating its activities. (iii) The coupling may perhaps be brought about by two factors, a tendency to defaecate just before the onset of headward irrigation and a 'fine adjustment' of the intervals between *i–d* outbursts to match the feeding rate. (iv) The factors controlling irrigation behaviour and the timing of defaecation in other circumstances are still obscure.

SUMMARY

The behaviour of lugworms in sand was studied by recording kymographically the water movements through their burrows and observing the timing of defaecation. The worms ranged in weight from 2·6 to 18·6 g. Three types of apparatus were used, differing in the amount of space allowed to the worms.

The worms often traced a characteristic pattern described elsewhere, consisting of regularly spaced diphasic excursions of the irrigation graph with a defaecation on the apex of each first phase. The interval between

excursions was about 15–20 min. for worms weighing about 3 g. and increased with size to about 40 min.

The worms sometimes traced similar periodic bursts of irrigation activity without defaecation.

Many of the tracings were confused in pattern. The worms often defaecated—sometimes sporadically, sometimes at regular intervals—in the absence of the characteristic diphasic excursions. Restriction of a worm to a small space appears to favour the production of confused tracings.

The relation between the irrigation patterns and the timing of defaecation is discussed.

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LUMINESCENCE IN POLYNOID WORMS

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(Plates I and II and Text-figs. 1–24)

Luminescence is well known in polychaete worms and occurs sporadically in unrelated species having different modes of life. Families containing luminescent species are Aphroditidae, Tomopteridae, Syllidae, Alciopidae, Chaetopteridae, Cirratulidae and Terebellidae. Among Aphroditidae luminescent forms are confined to subfamily Polynoinae,[1] and the response is limited to the dorsal scales which are a diagnostic feature of these animals. Bonhomme (1942) has recently demonstrated in polynoid worms that light production is intracellular. This contrasts with certain other polychaetes such as *Chaetopterus*, in which luminescence is extracellular and is due to the discharge of a luminescent secretion. The regulation of luminescence in *Chaetopterus* has recently been investigated in some detail (Hasama, 1941; Nicol, 1952*b*); and I considered that a study of the physiology of intracellular luminescence in polynoids would form an interesting comparison with that work (see Harvey, 1940, 1952, for general reviews of animal luminescence).

DISTRIBUTION OF LUMINESCENCE IN THE SUBFAMILY POLYNOINAE

Luminescence has been reported in the following polynoids, the nomenclature based on Fauvel (1923). Six of these species are illustrated in Text-fig. 1.

Luminescent species	Authority
Harmothoë impar (Johnston)	Fauvel, 1923; Bonhomme, 1942
H. impar (=*Evarne impar*)	McIntosh, 1900; Bonhomme, 1942
H. longisetis (Grube)	Fauvel, 1923
H. imbricata (L.)	McIntosh, 1872, 1900
H. imbricata (=*Polynoë cirrata*)	Kallenbach, 1883
H. spinifera Ehlers (=*Polynoë torquata*)	Jourdan, 1885
H. spinifera (=*H. torquata*)	Darboux, 1899
H. lunulata (Delle Chiaje)	McIntosh, 1877, 1900; Fauvel, 1923
H. lunulata (=*Polynoë lunulata*)	Bonhomme, 1942
H. aspera (Hansen) (=*Polynoë aspera*)	Khvorostànsky, 1892
Lagisca extenuata (Grube)	Darboux, 1899
Gattyana cirrosa (Pallas)	McIntosh, 1900
Lepidasthenia stylolepis Willey	Lloyd, 1907
Eunoë nodosa Sars (sp. ?)	McIntosh, 1872
Polynoë scolopendrina Savigny	McIntosh, 1872, 1900
Malmgrenia castanea McIntosh	Original
Acholoë astericola (Delle Chiaje)	Darboux, 1899; McIntosh, 1900; Kutschera, 1909; Dahlgren, 1916; Fauvel, 1923; Bonhomme, 1942
Lepidonotus squamatus (L.) (=*Polynoë squamatus*)	Dahlgren, 1916

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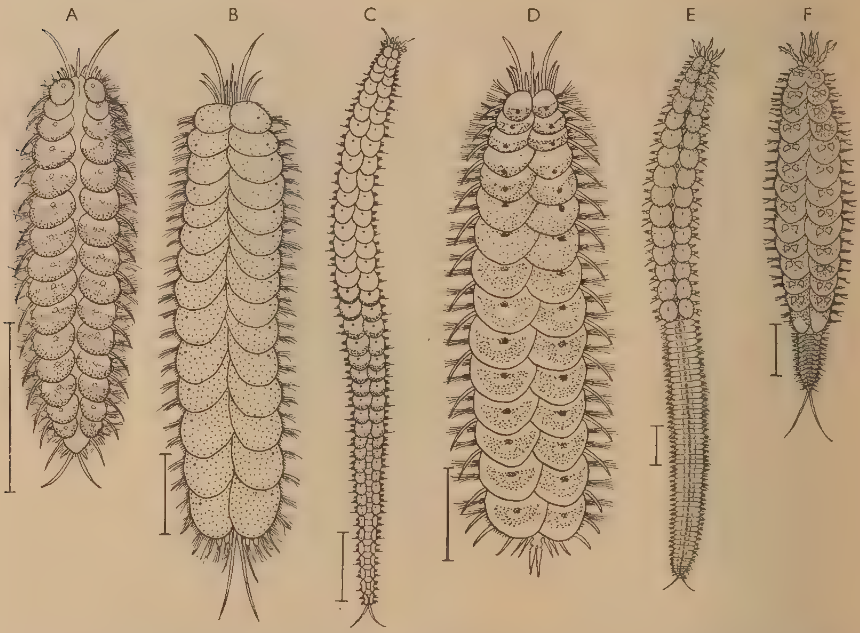
[1] McIntosh (1900) and Hartman (1938*a*, *b*) treat this subgroup as a separate family.

This list is certainly not final. It is also important to record species of polynoids that do not luminesce, in order to restrict the field of further search and investigation. Contrary to Dahlgren (see preceding list), the two species of *Lepidonotus*, *L. clava* (Montagu) and *L. squamatus* (L.) are not luminescent. This is noted by Jourdan (1885) and Bonhomme (1942) and I can confirm the observations of these two authors. *Halosydna gelatinosa* M. Sars and *Lepidasthenia argus* Hodgson are two other non-luminescent species.



Text-fig. 1. Luminescent species of polynoids, drawn from life. A, *Malmgrenia castanea*; B, *Gattyana cirrosa*; C, *Acholoë astericola*; D, *Harmothoë lunulata*; E, *Polynoë scolopendrina*; F, *Lagisca extenuata*. Scale 5 mm.

A number of authors have merely recorded luminescent genera, viz. *Polynoë*, *Harmothoë* and *Lepidonotus*. With repeated changes in nomenclature these observations are only of general value.

HISTOLOGY AND INNERVATION OF THE LUMINESCENT TISSUE

There is general agreement that luminescence in polynoids is confined to the scales which cover all or part of the dorsal surface, according to the species. These structures are disk-shaped, and are attached to the body above the parapodium by a relatively thin stalk or elytrophore. Fauvel (1923) notes that the elytra are inserted on segments 2, 4, 5, 7, 9, . . ., 23, 26, and subsequently on each successive third segment; segments without elytra have an elongated dorsal cirrus.

General Structure of the Elytrum

An elytrum is a thin disk covered externally by a cuticular layer which may be smooth or adorned on its superior surface with rugosities and papillae. Underneath the cuticle is a unicellular epidermis (or hypodermis) which is continuous except over the insertion of the stalk. Frequently the scale bears a pigment pattern which is due to the aggregation of dark granules in the epidermal cells.

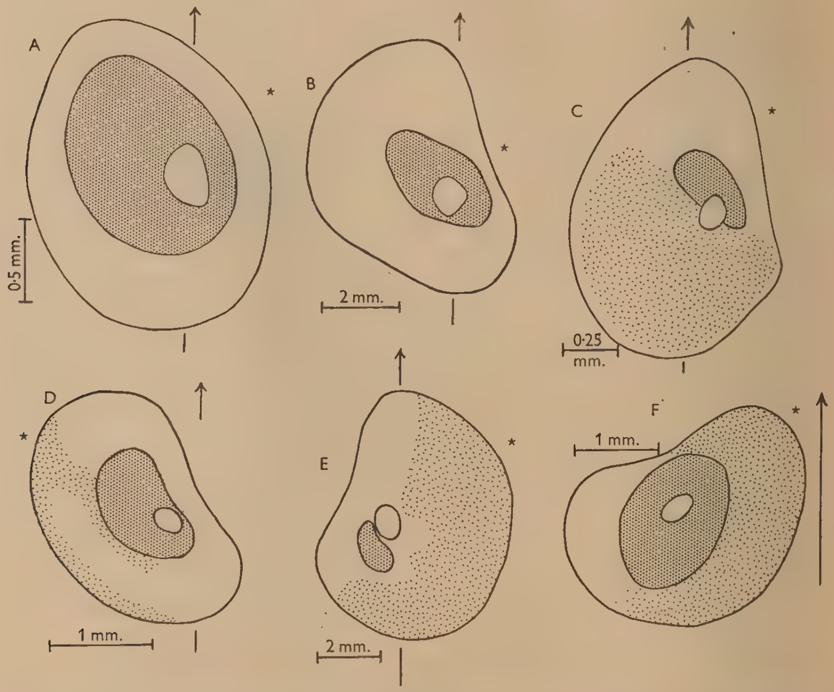
Extending vertically between the upper and lower surfaces of the scale is a strut-work of rather fine fibres of somewhat indeterminate nature. They are patently extracellular structures, presumably of connective tissue nature; their staining affinities are poor, but they are coloured by eosin. Since they are not concerned with luminescence it was unnecessary to analyse them further in this investigation. The meshwork of spaces among the fibres communicates with the general body cavity through the stalk. Well-developed muscles are present in the elytrophore, some of them inserted on the rim forming the junction between stalk and elytrum, but none extend into the body of the elytrum (Pls. I and II) (Darboux, 1889; Haswell, 1882; Jourdan, 1885; Pflugfelder, 1933).

In the light-producing species there is a layer of luminescent cells or photocytes on the lower surface of the scale. These cells are actually modified epidermal cells. Bonhomme (1942) has described in *Harmothoë lunulata* how the density of pigment is reduced in the epidermal cells of the upper surface over the region occupied by photogenic tissue. This restriction of pigment pattern is a general feature of luminescent species, and presumably is an adaptation which allows maximal emission through the upper surface of the scale (Text-fig. 2).

Innervation of the Elytrum

An easily recognizable nerve trunk ascends in the wall of the elytrophore, and on reaching the elytrum forms a well-demarcated nodal point from which nerves radiate out in all directions towards the periphery of the scale. These nerves divide, subdivide, and become attenuated towards their termination (Pl. I, fig. 1). Kallenbach (1883) refers to the node of nervous tissue above the elytrophoral stalk as a ganglion, but gives no details of the occurrence of nerve cells.

The nervous supply of the elytrum has been described and figured by Haswell (1882), Jourdan (1885), and Bonhomme (1942), to which I can add few additional observations. In favourable material (e.g. *Acholoë*, *Malmgrenia*), the nerves in the scales can be seen in the living state, but staining is necessary to bring out the finer nerves and fibres. Many of the smaller fibres terminate in relation to the papillae on the dorsal surface, or go to sensory terminals on the exposed margin of the scale. The dorsal papillae are generally believed to represent the loci of sensory structures. In the figures of Jourdan (1885, 1887), Bonhomme (1942) and others, the papillae are drawn with a central canal through which runs a fibre that is connected with an underlying group of ganglionic cells (Darboux, 1899; Haswell, 1882; Panceri, 1874).



Text-fig. 2. Outline drawings of the scales of polynoids to show the extent of the luminescent area. A, *Acholoë*; B, *Gattyana*; C, *Malmgrenia*; D, *Harmothoë*; E, *Lagisca*; F, *Polynoë*. Magnification as shown. The central clear area is the region of attachment of the elytrophore. The luminescent area is marked with mechanical shading. Pigmented areas are dotted. Direction cephalad is indicated by the arrow, and the external margin by an asterisk.

By means of silver impregnation (Bielschowsky method) Bonhomme has shown up the elytral nerves of *Harmothoë lunulata* (=*Polynoë lunulata*). Besides the sensory fibres and peripheral sensory cells he has discovered numerous efferent fibres which leave the main nerve trunks, and pass downwards to reach the photogenic cells, or even to penetrate into them and run between the secretory granules.

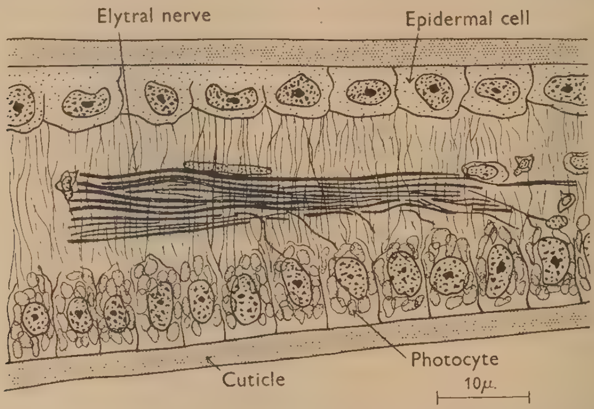
I can confirm these descriptions from preparations of *Acholoë astericola* treated with various nerve-stains. The scales of this species can be stained readily with supravital methylene blue, whereas other species (*Lagisca extenuata*, *Polynoë scolopendrina*) proved refractory. Clear pictures were obtained of nerves radiating outwards from a nodal point at the edge of the stalk, and ramifying over the expanse of the scale (Pl. I, fig. 1). This is the broad picture of the nervous supply. Silver-impregnation with Bodian's activated protargol has been tried with varied success on the scales of several species (*Polynoë*, *Acholoë* and *Lagisca*). In sections so treated the nerve trunk can be traced through the wall of the elytrophore into the centre of the elytrum. Here it gives rise to a well-marked ganglion consisting of a central mass of nerve fibres about which are grouped several distinct nerve cells (Pl. II, figs. 7, 8). From this central ganglion nerve trunks proceed peripherally through the centre of the scale (Pl. II, fig. 9). In their course the nerves give off fine nerve fibres which run obliquely or vertically downwards. In fixed material they are very fine, less than 1 μ in diameter. Fairly certainly, therefore, all the photogenic cells are innervated by fine nerve fibres, and these emerge from the nerve trunks which radiate outwards from the elytrophore like the spokes of a wheel. Consequently, the nerve fibres which occur in the elytrum are of two types, viz. sensory fibres, and efferent fibres supplying the luminescent cells.

Photogenic Tissue

The disposition and structure of the photogenic cells have been debated for some time, and the solution of this problem is necessary to understand the physiological processes involved in light production in polynoids. Kutschera (1909), followed by Dahlgren (1916), believed that the luminescent cells were grouped under the dorsal papillae of the elytra. Although unable to detect an extracellular secretion they supported the view that a photogenic material was discharged to the exterior through a canal in the papilla. These papillae, are however, probably receptor structures. The true photogenic cells form a glandular layer in the lower surface of the scale of luminescent species (Harvey, 1952).

The photogenic tissue in *Harmothoë spinifera* (= *Polynoë torquata*) has been described by Jourdan (1885). He showed that it occupied the region of the scale around the elytrophore and consisted of a layer of mucus-cells which replaced the epidermis over part of the lower surface of the scale. In his figure (3) masses of large granules are shown in the photogenic epithelial cells. Recently, the photogenic cells of *H. lunulata* have been described in more detail by Bonhomme (1942), who has made confirmatory observations on *H. impar* and *Acholoë astericola*. These cells are tall, polygonal in cross-section, and contain a large oval nucleus. Proximally the cells are filled with large oval or round granules which are closely packed together; distally, the granules decrease in size. Immediately under the cuticle the cell shows a basal cytoplasmic layer free of secretory granules and containing mitochondria. On histochemical grounds the granules were considered to be protein in nature, and were regarded as representing the luminescent material. The non-luminescent species of *Lepidonotus* lack a layer of corresponding photogenic tissue in their elytra.

I have prepared sections of the elytra of six luminescent species, namely *Acholoë astericola*, *Malmgrenia castanea*, *Harmothoë lunulata*, *Gattyana cirrosa*, *Polynoë scolopendrina* and *Lagisca extenuata*, and one non-luminous species, *Lepidasthenia argus*, and I can confirm Bonhomme's observations. The photogenic cells in the first six species listed above form a layer of unicellular epithelium on the lower surface of the scale near the elytrophore region, and extending a greater or lesser distance peripherally, according to the species (Pls. I and II). In all species the photogenic cells are packed with coarse granules, which are acidophilic after fixation in Bouin's and Helly's fluids, and stain well with eosin and orange G, and also with azocarmine (Text-fig. 3). Some measurements are as follows:



Text-fig. 3. Drawing of a vertical section through the elytrum of *Acholoë astericola*, showing photocytes, elytral nerves and connective tissue framework.

Species	Dimensions of cell (μ)	Size of granules (μ)
Lagisca extenuata	3–4 × 8–14	2·5–3
Polynoë scolopendrina	5–6 × 8–25	2–5
Acholoë astericola	3–4 × 8–18	2–5
Malmgrenia castanea	6–8 × 8	2
Gattyana cirrosa	10–18 × 4–8	2–7
Harmothoë lunulata	6–18 × 4–5	3–5

There are considerable specific differences in the area of the scale occupied by the photogenic tissue. This is brought out in Text-fig. 2, compiled from

Bonhomme and from original observations. Drawings (Text-fig. 2) illustrate the region of the scale which luminesces in different species. Reconstructions have also been made from serial sections of whole elytra of *Acholoë* to show the area of scale occupied by photogenic tissue, and reasonably close correspondence was found to exist between the area occupied by photogenic cells and the luminescent region.

It follows that luminescence is intracellular in polynoid worms, and takes place in a layer of eosinophilic photogenic cells lying on the lower surface of the elytrum. Presumably the granules occurring in these cells are the luminescent material.

Miscellaneous Observations on Luminescence in Polynoids

Early observations on luminescence in polynoids, reviewed by Darboux (1899), do little more than record its occurrence.

Luminescence is evoked by mechanical stimulation of the animal. *Acholoë astericola*, the species which has been most investigated, is very sensitive to mechanical disturbance; and, if the container is shaken or the animal touched, a few or many scales will luminesce, depending on the strength of the stimulus. Falger (1908) notes that when an animal is touched, the luminescent response proceeds anteriorly and posteriorly over the elytra from the stimulated region (Dahlgren, 1916; Darboux, 1899). When an animal is quickly cut into two, however, only the elytra of the posterior portion lighten, while the anterior half of the animal remains dark (Harvey, 1940, 1952; Kutschera, 1909). This kind of response is apparently common to all luminescent species, and has been observed in *Lagisca extenuata* and *Malmgrenia castanea*, as well as in *Acholoë astericola*. In *Polynoë scolopendrina* the posterior half of the body is devoid of scales, and when this region is transected there is no luminescent response. When, however, the anterior half of the body is cut across, the scales posterior to the cut become luminescent.

These observations show that luminescence in polynoids is under nervous control, since the excitation proceeds along the length of the body from the stimulated region. With suitable tactile stimulation the luminescent response proceeds both forwards and backwards along the worm. Consequently, pathways exist for the transmission of excitation anteriorly and posteriorly through the nerve cord. Since a sharp cut produces luminescence in the posterior region only, these nervous pathways are apparently functionally polarized, as has been observed in *Chaetopterus* (Nicol, 1952*a*, *b*). Possibly, strong stimulation is required to overcome the resistance in anteriorly directed nervous pathways, in which resistance stands at a higher level than in pathways leading posteriorly.

A notable feature of luminescence in polynoids is that the light appears in flashes or scintillations when the animal is irritated. This has been recorded for various species including *Harmothoë imbricata* and *H. lunulata* (Haswell, 1882; McIntosh, 1877, 1900). In *Acholoë astericola*, according to Falger (1908) and Kutschera (1909), the response lasts a few seconds and elytra light up intermittently for 20 or 30 times in quick succession.

The luminescent polynoids readily autotomize their elytra when irritated, and these separated scales continue to glow for some time when cast off from the animal. The nerve trunk is severed when a scale is cast off, and the excitation produced is sufficient, presumably, to start the scale flashing. Bonhomme observes that in the intact animal there are reflex pathways involving sensory receptors on the scale, the nerve cord, and efferent nerve fibres, all of which are concerned with mediating the luminescent response, but in the isolated scale only efferent fibres are in a position to be excited so as to lead to a response (Haswell, 1882; Jourdan, 1885; Kallenbach, 1883; McIntosh, 1900).

There are reports that the intact animal and isolated scales recover the ability to luminesce after stimulation when they are left for some time in sea water. Falger's results (1908) seem to indicate that recovery is a rather slow process.

Electrical stimulation has been employed by several workers (Bonhomme, 1942; Kutschera, 1909; Panceri, 1874). Falger (1908), using *A. astericola* found that direct current at make stimulated the animal to luminesce. By alternating the current he produced momentary flashes corresponding to each change of current, and finally, with faradic stimulation, he obtained prolonged responses in which the light appeared to flicker rapidly. These results are interesting in appearing to show that the response to a single shock is a quick flash. This kind of response should lend itself well to physiological analysis, and such a study was undertaken.

RESULTS OBTAINED WITH PHOTOELECTRIC RECORDING

Material and Methods

Recordings were made of luminescence in several species of polynoids which were collected in the Plymouth area: *Lagisca extenuata* (Grube), a common species on the shore, in dredgings, and among *Chaetopterus* tubes; *Harmothoë lunulata* (Delle Chiaje), commensal with *Arenicola marina* L., *Echinocardium cordatum* (Pennant), and *Leptosynapta inhaerens* (O. F. Müller); *Polynoë scolopendrina* Savigny, a commensal with *Polymnia nebulosa* (Montagu) which is most easily collected from among *Chaetopterus* tubes; *Acholoë astericola* (Delle Chiaje), a commensal living in the ambulacral groove of *Astropecten irregularis* (Pennant); *Gattyana cirrosa* (Pallas), occurring in the burrows of *Amphitrite johnstoni* Malmgren; and *Malmgrenia castanea* McIntosh, a commensal species found about the mouth region of *Spatangus purpureus* O. F. Müller (Text-fig. 1). The physiological basis of luminescence appears to be identical in all these species, and the results are considered together. *Acholoë asterícola* and *Polynoë scolopendrina* were used in most of the investigations, but sufficient records were obtained of luminescence in the other species to show that their behaviour was the same.

Each scale is an effector unit, and individual scales may be expected to show independent time courses of excitation and response. Therefore, to obtain quantitative data and to analyse the luminescent response, records must be secured from single scales. As brought out in the preceding section, the scales flash when detached from the body, and their luminescent ability thereby becomes reduced. To prevent this, and to obtain scales in a functionally fresh condition for examination, the animals were anaesthetized with an isotonic solution of magnesium chloride for 30 min. before handling them. With *Acholoë astericola*, which is very sensitive to tactile stimulation, and which luminesces strongly when an attempt is made to evict it from its host, it is best to anaesthetize starfish and worm together. This treatment narcotizes and immobilizes the worm, and the scales can be removed with fine scissors under a dissecting microscope without causing them to luminesce. Subsequently the magnesium should be washed out with several changes of sea water for an hour or more. Detached scales in sea water can be stored for a few days in a refrigerator (4–8° C.), until required for experimentation.

To record luminescence from a single scale, the scale was mounted on a pair of platinum electrodes lying in a moist chamber which was placed underneath a multiplier photocell. The arrangement is shown in Text-fig. 4. The light from the scale was focused on the photocathode of the photocell. The latter was connected through a direct coupled amplifier to a double-beam oscilloscope. Since there is much variation in the intensity of light produced by different species, it is necessary to regulate the degree of amplification, and this was achieved both by control of the amplifier, and by adjusting the voltage on the photocell by means of a potentiometer.

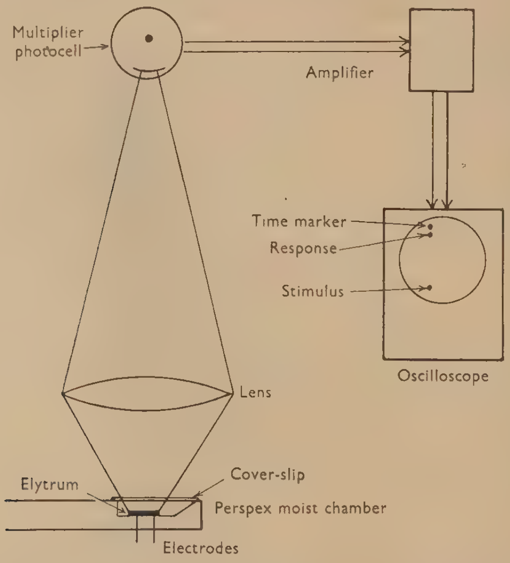
Electrical stimulation consisted of shocks of brief duration (capacitor discharges) delivered from an electronic stimulator which permitted independent control of frequency, voltage, and pulse duration.

Observations

Effect of Single Shocks (Acholoë)

When an elytrum is stimulated with a single shock it gives a bright luminescent response. This frequently takes the form of a series of flashes. An example of such a response is given in Text-fig. 5. The frequency of flashing is rapid at first, often at rates of 5 or more per sec. The pattern of flashing frequently takes the following form. After a rapid initial outburst lasting about a second, the rate of flashing settles down to a steady level of about 1 per sec. which continues for around 1 min., and then gradually begins to decline while the flashes themselves decrease in intensity. Text-fig. 10 shows a plot of flashing interval against time, taken from one of the photographic records, and bringing out certain of the features just described. In some records the rate of flashing increases instead of decreasing as the flashes die away, but this kind of response is less frequent.

A second characteristic of this repeated flashing or flickering is seen in a falling off of intensity. Initially the intensity of the flashes is relatively high, but thereafter the height of the separate responses decreases with time. This is shown in Text-figs. 5 and 6. When the rate of flashing is fairly rapid (intervals of less than 0·6 sec.), the light intensity does not return to zero between flashes (Text-fig. 5).



Text-fig. 4. Diagram of the apparatus used in recording the luminescent flashes of single elytra from polynoids. Not drawn to scale.

During repetitive flashing (induced by a single stimulus) the level of intensity reached by the first few flashes is often noticed to rise rapidly to a maximum, and then gradually decrease (Text-fig. 5). This effect shows in Text-fig. 8, which gives a plot of the light intensity of individual flashes against time. In the record from this elytrum the increase in intensity follows a linear course for the first three flashes, and then slowly decreases. Maximal intensity of flashes is reached in one-third of a second (third flash), and intensity of subsequent flashes falls to one-half the value of the maximal flash in 11 sec. (15 flashes). The rate of decay of intensity of subsequent

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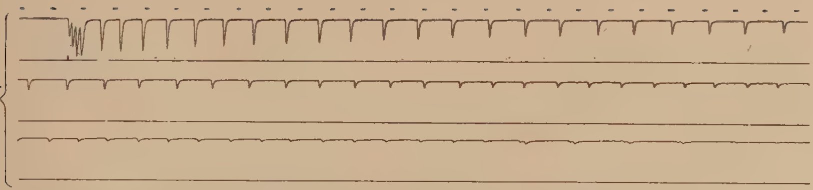

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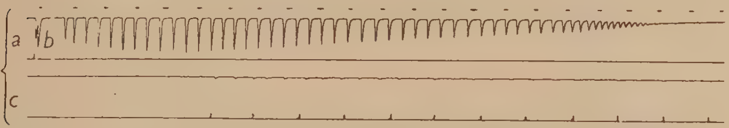

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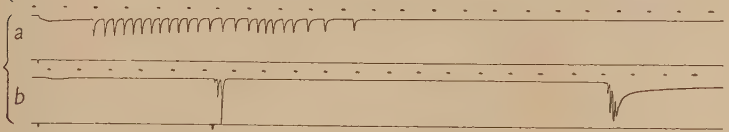

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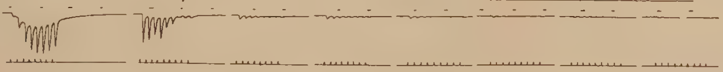

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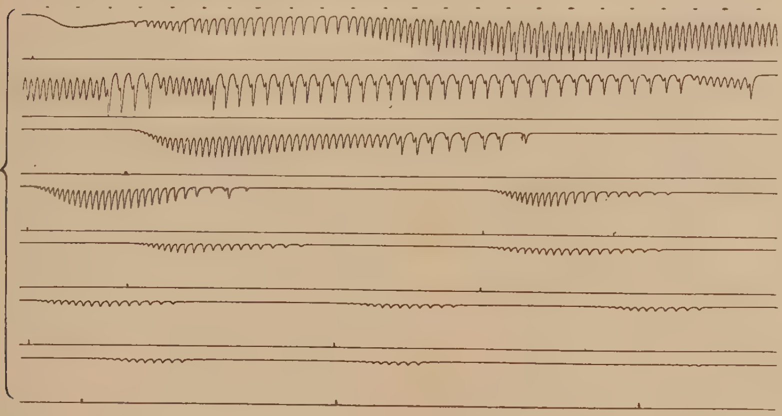


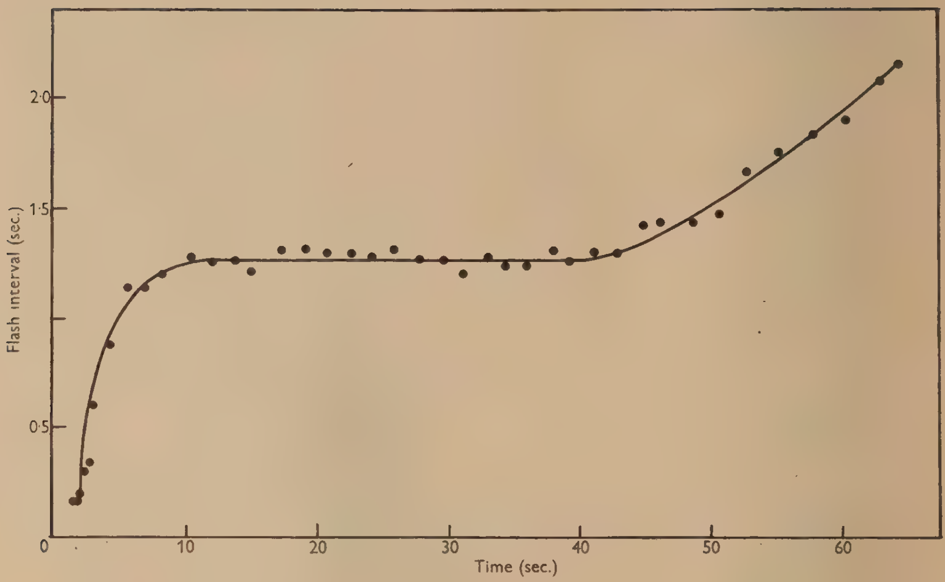
Text-fig. 5. Prolonged flashing from an elytrum of *Acholoë*, as the result of a single electrical stimulus. This is a continuous record. Time scale above, pips at 1 per sec. Deflexions downwards of the oscilloscope upper beam are records of the luminescent responses. Stimulus appears as pip on the lower line (oscillograph lower beam).

Text-fig. 6. Rhythmic flashes in an elytrum of *Acholoë* following the application of a single stimulus (*a*, *b*), and repeated stimuli (*c*). Stimulation frequency, 42 per min. in *c*. Time scale, 1 per sec. 1 sec. elapsed between records *a* and *b*; 1 sec. between *b* and *c*.

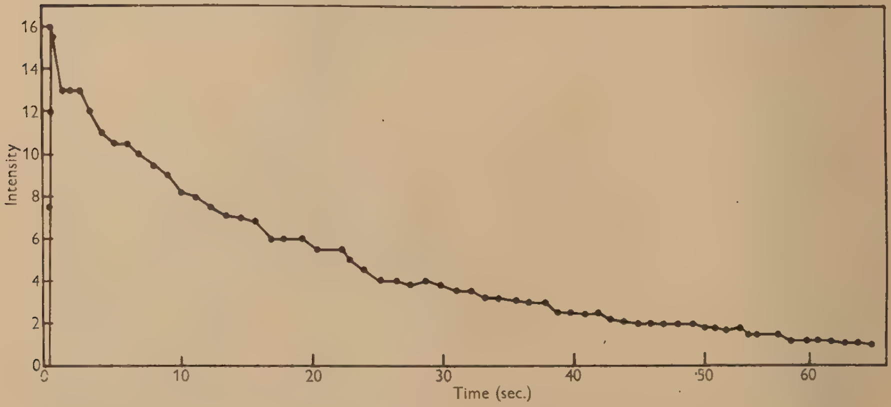
Text-fig. 7. Luminescent responses of the elytra of *Lagisca*: *a*, flickering induced by a single impulse; *b*, effect of two stimuli separated by an interval of 6 sec. Time scale 1 per sec. The small prolonged deflexion immediately following each stimulus may be due to some direct depolarization of the luminescent cells.

Text-fig. 8. Fatigue of the luminescent response of *Acholoë* as the result of successive bursts of electrical stimuli. These consisted of 7–8 shocks at a frequency of 6 per sec. Time scale 1 per sec.

Text-fig. 9. Rhythmic flashing of an elytrum of *Gattyana*. This is a continuous record showing consecutive periods of rhythmic flashing, each period of flashing being induced by a single shock. Time scale 1 per sec.



Text-fig. 10. A plot of flash-frequency against time for an elytrum of *Acholoë*. The data were derived from a record of a scale which flickered rhythmically for more than a minute following the application of a single electrical stimulus. Abscissae, time in seconds; ordinates, intervals between flashes in seconds.



Text-fig. 11. A plot of the light intensity of successive flashes in an elytrum of *Acholoë* against time. Rhythmic flashing was induced in the preparation from which this record was obtained by a single electrical shock. Light intensity in arbitrary units. Time in seconds. Each point on the curve represents the intensity of a separate flash.

flashes follows an exponential curve, and in about 1 min. is 10% of the maximal flash, and is barely measurable (Text-fig. 11). A curious feature of some records is that the flashes sometimes occur in bursts with considerable pauses in between. This is recorded in Text-fig. 6 (*Acholoë*) and in Text-fig. 7 (*Lagisca*). After a pause of as much as 10–14 sec. the scale may spontaneously start to flash once more, and continue until its powers of luminescence are exhausted.

Fatigue

Several factors are involved in determining the magnitude of the response, and one of the most important is the onset of fatigue. After a scale has been flashing for some time the flashes gradually become weaker. A highly excitable scale may continue flashing until it is nearly or completely exhausted, when the light becomes very weak or disappears. Subsequent stimulation then fails to evoke a response. Other scales, less excitable, respond with shorter periods of flashing to single shocks or brief bursts. These scales can be fatigued by repeating the stimulation at suitable intervals, when the consecutive flashes or groups of flashes gradually decrease in amplitude (Text-figs. 8 and 9). Isolated scales which have been stimulated to exhaustion show little or no recovery of luminescent ability when left overnight in sea water.

Since the luminescent response is an intracellular process, some oxidation of a cellular constituent must occur. Bonhomme (1942) has shown that after excitation there is no apparent decrease in the amount of intracellular secretory material in the photocytes when the luminescent response has run its course. Harvey (1926, 1952), moreover, has observed that fresh elytra of *Acholoë* are non-fluorescent, but once they are stimulated and luminescence has subsided, they display marked yellowish fluorescence. It appears, therefore, that some waste or end-product of the luminescent reaction is strongly fluorescent, and by this means a transformation of the intracellular constituents concerned with luminescence can be detected.

There are suggestions by earlier workers (p. 72) that polynoids recover the ability to luminesce after a period of rest, but since no comparative measurements were made these statements are rather indefinite. My observations show that recovery is a very slow process in isolated elytra. Apparently, then, either the luminescent reaction is irreversible, or reduction of the oxidized photogenic material takes place very slowly within the photocytes. Repeated flashing, therefore, progressively exhausts the store of potentially luminescent material until the scale becomes dark, and renewal of this store is dependent upon protracted chemical transformations within the photocytes. Isolated scales, of course, are deprived of trophic supplies from the animal, and this lack may restrict the energy sources available for reconstitution of the photogenic material.

It is of interest to compare fatigue of luminescence in different forms. In glowworms and fireflies (Lampyridae), luminescence is also an intracellular phenomenon, but these animals are able to produce light for many hours and do not show fatigue. On the other hand, various marine Crustacea which possess photophores, e.g. *Nyctiphanes*, *Sergestes*, fatigue readily as the result of repeated stimulation (Terao, 1917; Vallentin & Cunningham, 1888). I have myself shown (Nicol, 1952*a*), that the luminous polychaete *Chaetopterus variopedatus* discharges a luminescent secretion into the surrounding sea water, and when it is repeatedly excited the amount of secretion, and consequently light, rapidly diminishes with each period of stimulation. These few selected observations demonstrate that there exists extensive variation in the ability of different animals to maintain sustained or recurrent luminescence at a high level. This is of first importance in considering the role of luminescence in the life of the animal, and in seeking to unravel the physiological events involved in the control of light-production in each species.

Most biochemical work on luminescent substances has been done on the photogenic secretion of *Cypridina* (see Harvey, 1952, for a review of the relevant literature). *Cypridina* luciferin has been prepared in a highly purified state and this substance is known to undergo an irreversible oxidation when it is converted into oxyluciferin, with the release of light energy, through the activity of luciferase. These exact and detailed studies afford some ground for believing that in *Chaetopterus*, and possibly in polynoids, we are also dealing with an irreversible oxidation of photogenic material.

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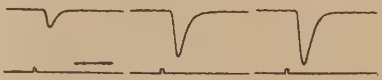

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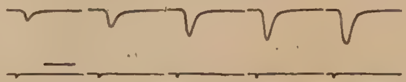


Text-fig. 12. Single flashes in an elytrum of *Acholoë*, induced by single impulses. Stimulating shock shown on lower line. There was an interval of 3 sec. between the first and second stimuli, and 5½ sec. between the second and third. Time scale, shown by the horizontal line at the bottom left, 100 msec.

Text-fig. 13. Luminescent responses of *Polynoë scolopendrina*. This record shows consecutive single flashes, 1 per stimulus, induced by a slow rate of stimulation (1 per sec.). Time scale below, 100 msec. Note the progressive increase in the height of consecutive flashes.

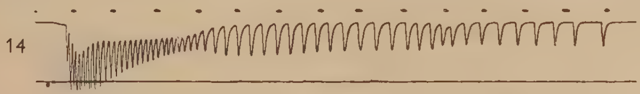
Time Characteristics of Single Flashes

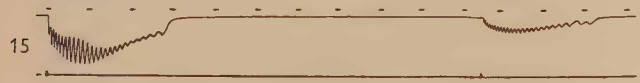
Favourable records of single flashes photographed at fast camera speeds permit an analysis of the temporal relations of the luminescent response (Text-fig. 12). In *Acholoë* the luminescent response (flash) begins 18–20 msec. after the stimulus, rises to a peak in 18–23 msec. from initial deflexion, and returns to base-line (zero intensity) from maximal deflexion in 45–80 msec.

Mean values are 19 msec. for latent period and 83 msec. for total flash duration. Time for half decay from peak intensity ranges from 11 to 19 msec. The response times for *Polynoë* scales are somewhat similar. The latent period varies from 13 to 21 msec., peak intensity is reached 26–30 msec. after initial deflexion, and the response is finished 108 msec. after maximal intensity is reached. Half decay from maximal deflexion occurs in about 17 msec. (Text-fig. 13).

Comparison of the Luminescent Responses of Different Species

The luminescent responses of different species of polynoids appear to conform to the same pattern as that found in *Acholoë*. In *Lagisca extenuata* (Text-fig. 14), *Harmothoë lunulata* (Text-fig. 15), *Malmgrenia castanea* (Text-fig. 16), *Polynoë scolopendrina* (Text-fig. 17), and *Gattyana cirrosa* (Text-fig. 9), a single shock either evokes one flash or initiates flickering, according to the condition of the elytrum. Rates of rhythmic flashing in the various species show the following ranges: *Lagisca*, 9–2 per sec.; *Polynoë*, 9–4 per sec.; *Harmothoë*, 9–1 per sec.; *Malmgrenia*, 8–1 per sec.; *Gattyana*, 9–2 per sec. Rates of flashing in *Acholoë*, for which a large series of records are available, vary from 18 to 1 per sec., but usually lie below 10 per sec. In general, it may be said that the scales of all species examined begin flashing at a rate of about 9 per sec., and the rate progressively falls off to a frequency of about 1 flash per sec. Other features common to all species are the gradual build-up in intensity of the first few flashes, and, once maximal flash intensity is reached, the progressive diminution in intensity of successive flashes as fatigue sets in.





Text-fig. 14. Luminescent responses of an elytrum of *Lagisca*, showing rhythmic flashing induced by a single electrical stimulus. Time scale, 1 per sec.

Text-fig. 15. Rhythmic flashing in a scale of *Harmothoë lunulata* as the result of a single electrical stimulus. Time scale, 1 per sec.

Effect of Stimulating the Nerve Cord

Most experiments have been made, for convenience, on isolated scales. To get additional information about the nature of nervous control of the luminescent response, some additional experiments were tried on *G. cirrosa* in which the nerve cord was stimulated. The preparations consisted of pieces of the body amounting to three or four segments. A single scale was left on each piece, and the body-wall was slit longitudinally along the ventral surface on one side of the nerve cord opposite to that bearing the scale. The preparation was then mounted on a glass plate, with the elytrum downwards, above the photocell. A micromanipulator was used to place a pair of needle electrodes on the nerve cord in that segment bearing the scale. Conveniently, the entire central nervous system, brain and nerve cord, in all polynoids is

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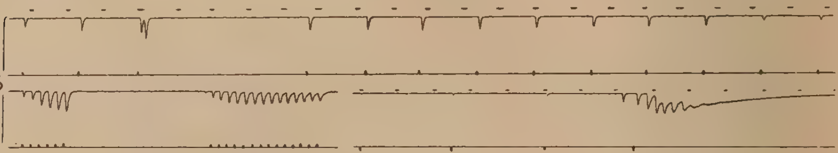

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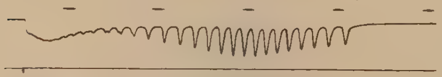

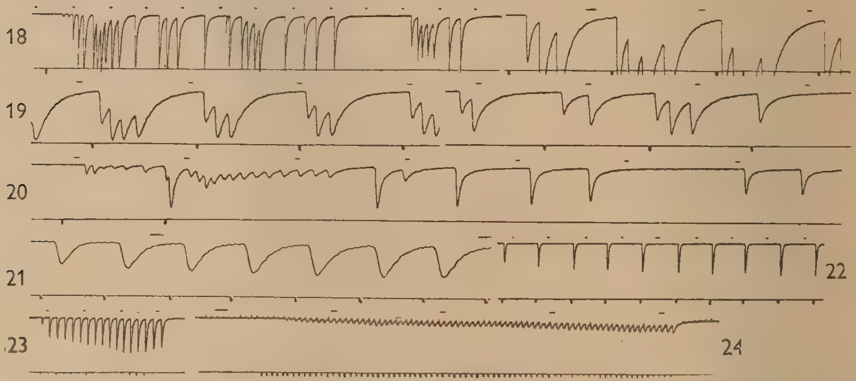

Text-fig. 16. Single flashes and repeated flashes from an elytrum of *Malmgrenia* as the result of electrical stimulation. Time scale, 1 per sec.

Text-fig. 17. Luminescent responses from a scale of *Polynoë*. Rhythmic flashing induced by a single stimulus. Time scale, 1 per sec.

Text-figs. 18–24. Luminescent responses from an elytrum of *Gattyana çirrosa* resulting from stimulation of the ventral nerve cord. Time scale in all records, 1 per sec. Fig. 18: rhythmic flashing induced by a single electrical shock. Fig. 19: rhythmic flashing in response to stimulation at a frequency of 72 per min. Fig. 20: rhythmic flashing induced by a pair of stimuli. Fig. 21: burst of shocks at a frequency of about 5 per sec. The response takes the form of a single flash for each stimulus. Fast paper. Figs. 22 and 23: responses to bursts of stimuli at 1 and 5 per sec. respectively. Note the gradual increase in intensity of successive responses. Fig. 24: burst of stimuli at 20 per sec. The rate of flashing follows that of stimulation.

easily recognizable in being coloured bright scarlet and visible through the body-wall. The colour is presumably due to haemoglobin, as in *Aphrodite* (see Fordham, 1925). Shocks of minimal strength (threshold) were used in order to restrict the stimulation to the nerve cord. The strength of shocks required to stimulate the nerve cord was found empirically to be only a small fraction of that necessary to stimulate an isolated scale. Consequently, the possibility of a spread of the stimulus to the scale itself can be excluded in these experiments.

As in isolated scales, a single shock applied to the nerve cord usually evokes a series of rhythmic flashes (Text-fig. 18). A gradual increase in the intensity of the first few consecutive flashes appears in some of these records (Text-figs. 18 and 19), and some preparations showed considerable irregularity in the pattern of responses, both in height and timing (Text-fig. 18 and 20). The irregularity which appears in some of the *Gattyana* records suggests strongly that several neuro-effector units are present, each one consisting of a group of photocytes supplied by one neurone, and that these units may be responding at slightly different rates, and be out of phase.

Stimulation at higher frequencies is shown in Text-figs. 21 and 24. Above 1 per sec. the flashes often follow the stimuli faithfully, and successive flashes increase gradually in intensity.

The conclusion from these observations is that the luminescent responses of the elytrum are controlled by the nervous system; and that efferent pathways exist from the nerve cord to the photocytes in the elytrum. It is significant also that a single impulse from the nerve cord will cause repetitive flashing in the elytrum, possibly through the intermediation of the ganglion situated in the latter structure.

DISCUSSION

The most striking feature of the luminescent response of polynoids is that it usually takes the form of quite rhythmic flashes. The evidence presented shows that the response is under nervous control and is normally evoked by tactile stimulation (touch or mechanical disturbance). A single electrical shock, mechanical injury to an isolated scale, and autotomy of the scale result in rhythmic flashing. Without further information there is little value in speculating on the mechanism responsible for this effect, but suggestions that would be worth further exploration are injury potentials from severed nerve fibres, rhythmic oscillations in the soma of ganglion cells supplying the photocytes, and reverberation of impulses in some system of closed neuronal arcs in the elytrum. Since electrical stimulation of the nerve cord as well as of the isolated elytrum causes rhythmic flashing, it appears that this type of response is characteristic of the normal animal, and a mechanism involving the elytral ganglion may be operating.

A second point of interest, revealed by the photographic records, is the gradual increase in intensity of the first few discrete consecutive flashes in a rhythmic response, or under repetitive stimulation. There is good evidence here for a process of facilitation taking place at the neuro-effector junctions, and closely analogous in many respects to the corresponding phenomenon described in neuro-muscular physiology. The quick flashes, closely following nervous stimulation, and the sharp records which can be obtained are very suitable for physiological analysis, and further studies are being pursued.

The regular rhythmic flashing, often lasting for a minute or more, and leading to exhaustion of the luminescent material, may in itself have marked functional significance. A mechanism of this kind allows discontinuous light emission over a much longer period of time than would be effected by continuous emission at the same intensity, and is a more economical method of utilizing a given amount of photogenic material. It can be argued that rhythmic flashes at rates of 10 to 1 per sec. are adequate to attract and secure the attention of another animal, and thereby fulfil their intention. A flickering light also may be more effective as a photic stimulus than a continuous light; but here experimental confirmation is required.

Several authors have commented on the possible significance of luminescence in polynoids, and have suggested that it acts in the nature of a sacrifice lure (Dahlgren, 1916; Haswell, 1882; Kutschera, 1909). This viewpoint is based on the behaviour of the animal. As noted previously, only the posterior half of the animal luminesces after transection. The anterior half is capable of directed locomotory movements, whereas the posterior half shows writhing movements but retains its same relative position. It has therefore been argued that when a polynoid is attacked and part of it is seized, the luminescing posterior region holds the attention of the predator, while the anterior region has the opportunity of escaping. Dahlgren (1916) presents an illustration by Horsfall which depicts such a situation. The anterior region of a polynoid is, of course, capable of regenerating the missing segments. Active, luminescent polynoids also possess a well-developed muscular mechanism for autotomizing their scales and these are readily cast off when the animal is irritated. Such isolated scales have been observed to flash rhythmically when removed from the body, and could well serve the function of a sacrifice lure. The reaction time is also fairly rapid, the first flash occurring in about $\frac{1}{50}$th of a second after stimulation of the nerves, and this would make the mechanism quite efficient. Since the scale soon exhausts itself after a series of flashes, the mechanism is evidently designed to function only at infrequent intervals. Such a theory, of course, must remain speculative until direct evidence is adduced to substantiate it.

It is of some interest to speculate on the evolution of luminescence among polynoids. In the family Aphroditidae, luminescence is confined to the subfamily Polynoinae, and to certain genera of the latter only. Known luminescent genera are *Harmothoë*, *Lagisca*, *Polynoë*, *Acholoë*, *Eunoë* and *Malmgrenia*. Except for the species *Lepidasthenia stylolepis*, *Lepidasthenia* and *Lepidonotus* are non-luminescent. Discussing the affinities of the Polynoinae, Darboux (1899) groups together the first four genera as closely related and probably derived from some common form, such as *Harmothoë*. Evidence suggests that they may have evolved independently of a second group including *Lepidasthenia* and *Lepidonotus*. Except for *Lepidasthenia stylolepis*, luminescence would seem to have appeared in some ancestral form common to one group of polynoids, and has been retained by its modern derivatives. This is a simpler explanation than one which assumes that luminescence has evolved on a number of separate occasions in this subfamily. Luminescence may, of course, have been lost by certain species, for example, *L. argus*.

SUMMARY

Six species of luminescent polynoids of the Plymouth fauna have been studied, namely *Lagisca extenuata*, *Gattyana cirrosa*, *Harmothoë lunulata*, *Polynoë scolopendrina*, *Acholoë astericola* and *Malmgrenia castanea*. Their scales are luminescent, and the light is produced by granular eosinophilic photocytes, which form a unicellular layer on the lower surface of the scale. The nervous supply of the elytrum is described, and the luminescent response is shown to be under nervous control. Luminescent responses from all six species have been recorded by the use of a photomultiplier cell and oscilloscope. The normal response has been found to consist of a series of rhythmic flashes, from 9 to 1 per sec., lasting up to 1 min. Some characteristics of the luminescent responses are given, and the part they may play in the normal life of the animal is discussed.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Scale of *Acholoë* stained supravitally with methylene blue (scale, 0·5 mm.).

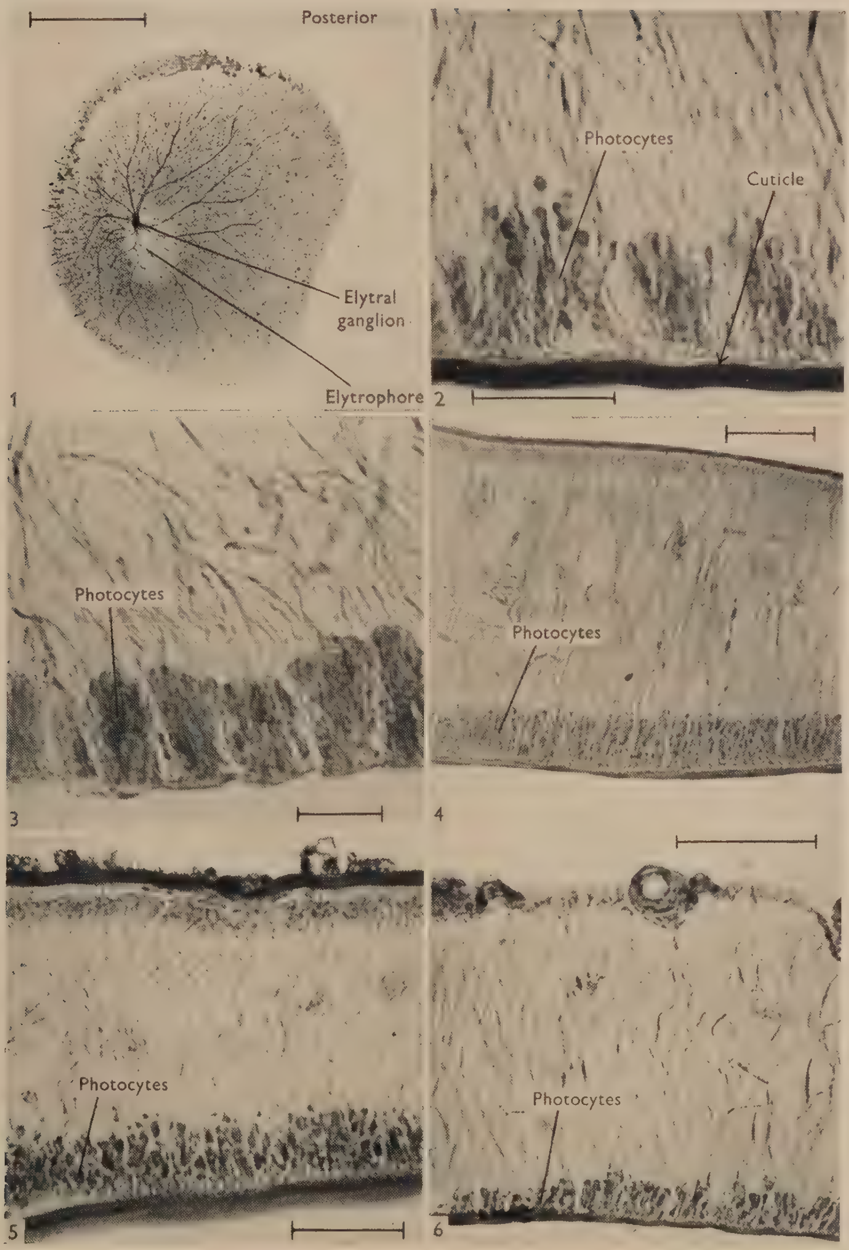
Figs. 2–6. Horizontal sections through elytra to show internal structure. Fig. 2: *Harmothoë* (25 μ). Fig. 3: *Gattyana* (10 μ). Fig. 4: *Polynoë* (25 μ). Fig. 5: *Acholoë* (20 μ). Fig. 6: *Lagisca* (50 μ). Heidenhain-azan and haematoxylin-eosin.

PLATE II

Fig. 7. Section through elytrophore and scale of *Malmgrenia* (Heidenhain-azan). Scale, 100 μ.

Fig. 8. Section through the central region of the elytrum of *Acholoë*, to show elytral ganglion and origin of an elytral nerve. (Bodian silver stain). Scale, 20 μ.

Fig. 9. Section through an elytrum of *Acholoë* to show elytral nerve and photogenic cells. (Bodian silver stain.) Scale, 20 μ.


Posterior
Elytral ganglion
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Photocytes
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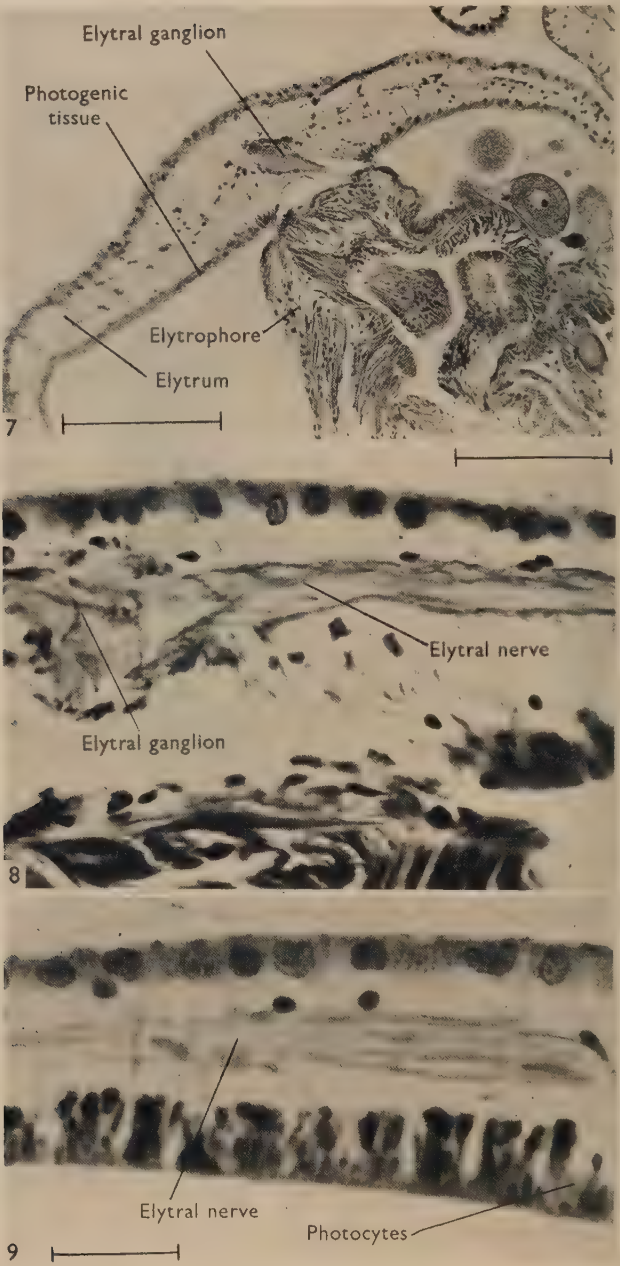
Figs. 1–6.


Elytral ganglion
Photogenic tissue
Elytrophore
Elytrum
7
Elytral nerve
Elytral ganglion
8
Elytral nerve
Photocytes
9

Figs. 7–9.

ON THE BIOLOGY OF *GLOSSUS HUMANUS* (L.) (*ISOCARDIA COR* LAM.)

By G. Owen

The Marine Station, Millport, and the Department of Zoology,
University of Glasgow

(Text-figs. 1–9)

Glossus humanus (L.), the only living representative of the genus, is a large eulamellibranch living in Western European seas and the Mediterranean. Nicol (1951) has reviewed the geological history and geographical distribution of the species, but little is known of its structure and habitat. Specimens were obtained from the Clyde Sea Area by use of an Agassiz trawl in depths of over 50 m. in the channel between the islands of Arran and Bute. Work on these specimens was carried out at the Marine Station, Millport, and the Zoology Department of the University of Glasgow.

I wish to express my gratitude to Prof. C. M. Yonge, F.R.S., for his encouragement and guidance during the course of this work and also to the Director and Staff of the Millport Laboratory for assistance in many ways. I am indebted to Dr Vera Warrender for the drawing of the shell reproduced in Fig. 1. Acknowledgement is also due to the Development Commission for financial assistance.

THE SHELL

The shell in *G. humanus* is equivalve, inequilateral and markedly globular with the umbones spirally enrolled and directed anteriorly. Many of the generic and specific names that have been applied to this species result from the resemblance of the shell, when viewed anteriorly, to the mammalian heart (Fig. 1).

In the Lamellibranchia, the direction of growth at any region of the valve margins may be resolved into: (*a*) a radial component radiating from the umbone and acting in the plane of the generating curve; (*b*) a transverse component acting at right angles to the plane of the generating curve; (*c*) a tangential component acting tangentially to, and in the plane of, the generating curve (Owen, 1952). The radial component is always present and affects the form of both valves. In the primitive bivalve, the normal axis was probably a plano-spiral and the form of the shell the resultant of radial and transverse components. In *G. humanus*, however, the normal axis (Fig. 2, NA) is a turbinate spiral, indicating that the form of the shell is the resultant of radial (R), transverse and tangential (T) components.

In genera where the tangential component is absent (e.g. *Tellina*), the cardinal teeth radiate across the hinge plate, increasing slowly in all dimensions with growth. But in *G. humanus*, the mantle isthmus (Owen, Trueman & Yonge, 1953) is continuously displaced posteriorly due to the effect of the tangential component on the mantle edge (Fig. 2). As a consequence, the cardinal teeth of both valves are long and extend almost parallel to the sides of the hinge plate (CC, ACR and PCR). The anterior lateral teeth are much reduced or absent (Lamy, 1920) while the posterior lateral teeth (PLR) are elongate, since in this region the tangential component, by displacing the secreting surfaces posteriorly, reinforces the effect of the radial component.

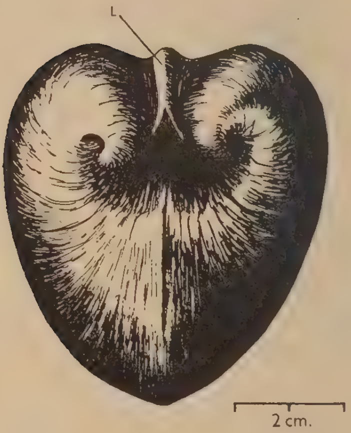


Fig. 1.

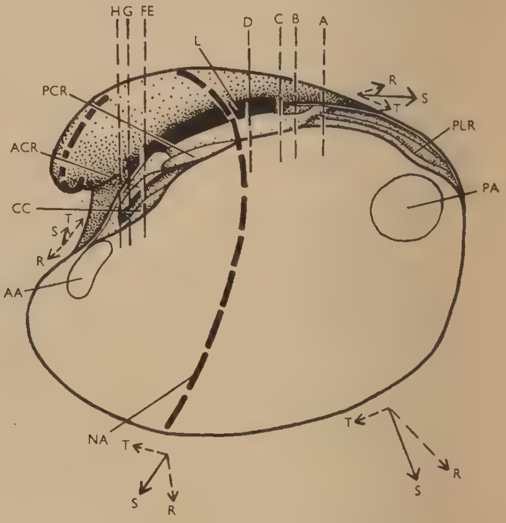


Fig. 2.

Fig. 1. *G. humanus*, the shell viewed anteriorly. L, ligament.

Fig. 2. *G. humanus*, the right valve showing the dentition of the hinge plate. AA, anterior adductor muscle scar; ACR, right anterior cardinal tooth; CC, central cardinal tooth; L, ligament; NA, normal axis; PA, posterior adductor muscle scar; PCR, right posterior cardinal tooth; PLR, right posterior lateral tooth; R, radial component; S, direction of growth at the mantle/shell margin; T, tangential component. The letters A–H refer to positions of transverse sections drawn in Fig. 3. Sections 3E and F are represented by a single line.

The description of the hinge teeth given by Jeffreys (1863) is confusing, for the dentition of the right valve is described as that of the left and vice versa.

The tangential component also affects the anterior region of the external opisthodetic ligament (Owen, 1953). When there is no tangential component affecting the form of the valves, as in *Glycymeris*, growth of the mantle/shell (Yonge, 1953) anterior to the umbones takes place anteriorly, i.e. away from the umbones. In *Glossus humanus*, however, as shown in Fig. 2, the radial (R) and tangential (T) components anterior to the umbones are opposed. Owing to the greater magnitude of the tangential component at this region, growth of the mantle/shell margin is in a posterior direction between the umbones, causing them to be increasingly separated as growth proceeds. This separation of the umbones results in a progressive splitting of the anterior region of the ligament, the split portions extending under the spirally twisted umbones (Fig. 1, L).

Covering the external surface of the shell is a reddish brown periostracum which, except at the anterior ventral to the umbones, is produced into numerous radiating rows of small bristle-like projections ('cilia' of Forbes & Hanley, 1853). The shell is thin and remarkably light in weight, the smaller specimens being extremely fragile. Bøggild (1930) has described the detailed structure of the layers composing the shell valves. Internally the valves have a smooth porcellaneous appearance while the pallial line is poorly defined. The posterior adductor muscle scar (Fig. 2, PA) is larger than the deeper anterior scar (AA). This deeper insertion of the anterior adductor muscle is possibly due to the lower rate of growth of the anterior region of the shell. The rate of migration of the anterior adductor muscle is, therefore, considerably less than that of the posterior adductor, while the rate of deposition of the inner layers of the shell is presumably equal for both regions.

THE LIGAMENT AND MANTLE ISTHMUS

In describing the structure of the ligament in *G. humanus* the nomenclature suggested by Owen *et al.* (1953) has been used. The relationships of the mantle, ligament and valves are shown in Fig. 3 (A–H). These figures form a series taken from posterior of the ligament to a region near the umbones as shown in Fig. 2. The interpretation of the structure and formation of the ligament will be more readily understood by comparing the sections shown in Fig. 3 with the diagrammatic representation of the ligament shown in Fig. 4.

Fig. 3A represents a section through the valves and mantle between the siphons and the posterior end of the ligament. Here, as shown in Fig. 4B, the inner lobes (FI) are fused, forming a ridge of tissue lying between the middle lobes (M). The periostracum (P) is secreted from the groove between the middle and outer (O) lobes. Anteriorly (Fig. 3B), the periostracum is present as a single delicate thread and the middle lobe (M) is represented by a median ridge of tissue between the two outer lobes. This appearance in transverse section of a single periostracal thread and of a single middle lobe does *not* represent a fusion of the periostracal grooves and of the middle mantle lobes. As will be seen from Fig. 4A, the transverse section represented in Fig. 3B passes through the periostracal groove (PG) and the middle lobe (M) of the mantle edge at the depth of the posterior embayment between the

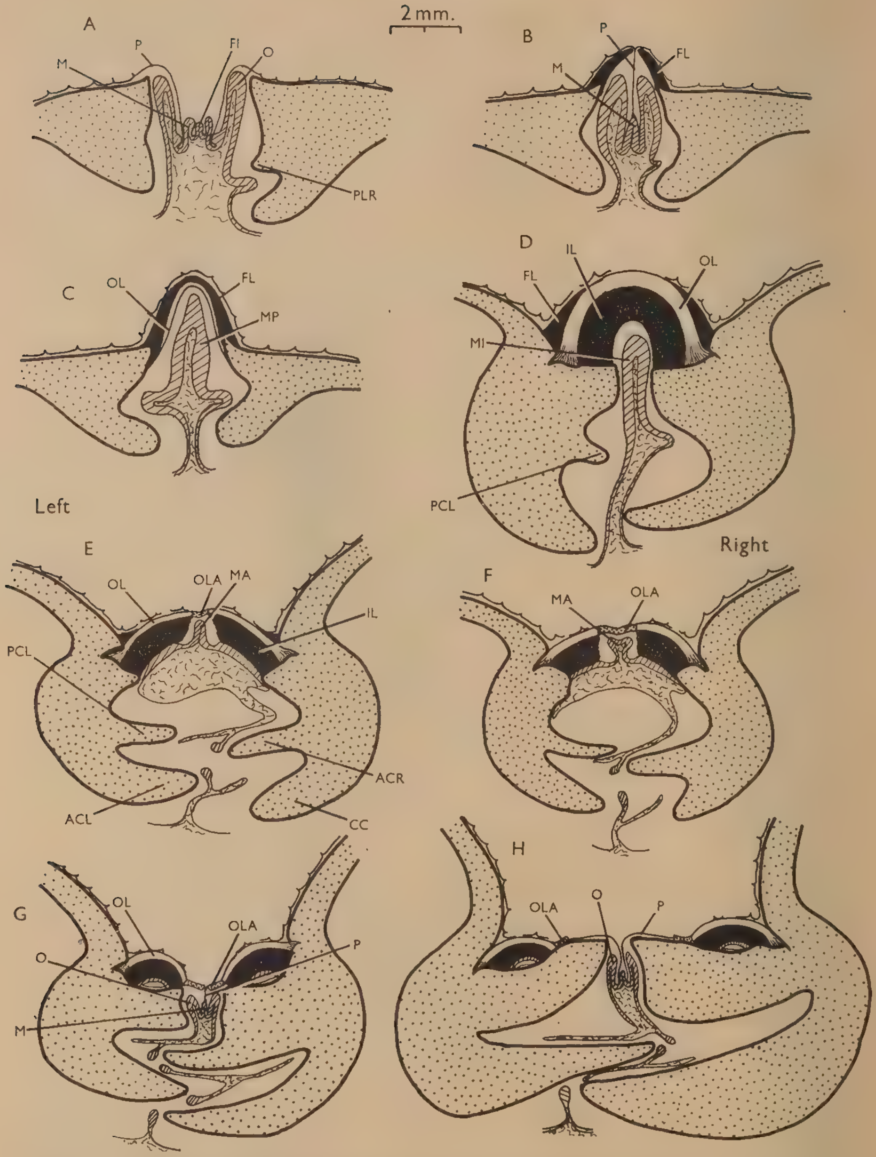

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Fig. 3. *G. humanus*, diagrams representing transverse sections through the ligament, valves and mantle. The positions of the sections are shown in Fig. 2. ACL, left anterior cardinal tooth; FI, fused inner lobes; FL, fusion layer; IL, inner layer; M, middle lobe; MA, mantle edge anterior to the mantle isthmus; MI, mantle isthmus; MP, mantle edge posterior to the mantle isthmus; O, outer lobe; OL, 'outer' layer; OLA, anterior 'outer' layer; P, periostracum; PCL, left posterior cardinal tooth. Other lettering as before.

two lateral lobes of the mantle. The outer lobes secrete on each side a flange of conchyolin-like material (FL) attached laterally to the edges of the valves. Anteriorly (Fig. 3C), this secretion forms an inverted V-structure linking the two valves. This fusion layer (Owen *et al.* 1953) is secreted by the fused outer lobes posterior to the mantle isthmus (Fig. 4, FO). At the posterior end of the mantle isthmus, the mantle edge (Fig. 3C, MP) secretes additional layers of horny material (OL) on the inner surface of the fusion layer. This horny material is the 'outer' layer of the ligament (Fig. 4B). Fig. 3D represents a section through the mantle isthmus (MI) and shows the bulky inner layer (IL) secreted by the epithelium of the isthmus and characteristic of the ligament of most lamellibranchs (Owen *et al.* 1953).

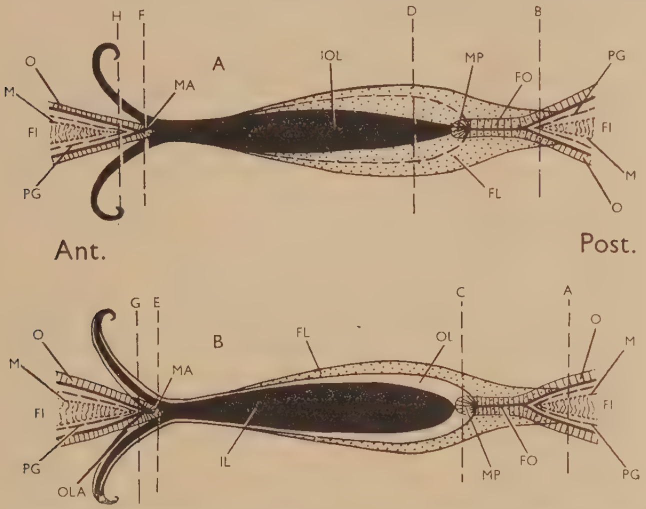

Fig. 4. *G. humanus*, diagrammatic representation of the ligament, A, viewed dorsally, B, horizontal longitudinal section. FO, fused outer lobes; IOL, 'outer' and inner layers of ligament; PG, periostracal groove. Other lettering as before. The letters A–H refer to the sections drawn in Fig. 3.

Although the sections shown in Fig. 3(A–D) were taken from different regions of the same specimen they could be interpreted equally well as having been taken, a constant distance posterior to the umbones, from different specimens at various stages of growth. Fig. 3A would then represent the youngest growth stage and Fig. 3D the oldest growth stage. During growth, the mantle isthmus extends posteriorly below the periostracum, fusion layer and 'outer' layer. In the region of the isthmus, the ligament therefore consists of a superficial periostracum, a fusion layer, an 'outer' layer and a bulky inner layer (Figs. 3D and 4B). The fusion layer becomes split in the mid-line above the posterior region of the inner layer (Fig. 4A).

The anterior region of the ligament in *G. humanus* is progressively split along the mid-line as growth proceeds. This split first appears in the inner layers but, as shown in Fig. 3E, soon extends to the outer layers of the ligament. A ridge of epithelium, representing the edge of the mantle anterior to the mantle isthmus (Fig. 4B, MA), secretes a layer of horny material (OLA) connecting the split portions of the 'outer' layer. Anteriorly (Fig. 3G), the periostracum (P) is present as a single thread while the middle lobe (M) is represented by a median ridge of tissue between the outer lobes (O). As at the posterior end of the ligament, this appearance of the periostracum and of the middle mantle lobe is *not* the result of fusion. As will be seen from Fig. 4B, the transverse section represented in Fig. 3G passes through the periostracal groove (PG) and middle lobe (M) of the mantle edge at the depth of the anterior embayment. The horny layer (OLA) produced at the anterior end of the ligament is split, the periostracum being attached laterally to the broken edges (Fig. 3G). The periostracum produced at this anterior region differs from that over the remainder of the shell in that it is not produced into bristles or 'cilia'. Finally, the mantle margin at the anterior end of the ligament has the appearance shown in Fig. 3A.

Apart from the effect of the tangential component upon it, the ligament in *G. humanus* is that of a typical bivalve possessing an external opisthodetic ligament. Fusion of the outer mantle lobes posterior to the mantle isthmus involves the secretion of a fusion layer representing a secondary extension of the ligament (Owen *et al.*, 1953). The mantle edge posterior to the isthmus secretes the 'outer' layer of the ligament. Since growth of the mantle isthmus is in a posterior direction only, this 'outer' layer produced posterior to the isthmus forms the functional 'outer' layer of the ligament. The horny material (OLA) or 'outer' layer secreted by the mantle edge (MA) anterior to the isthmus is progressively split as growth proceeds due to the effect of the tangential component on the mantle/shell margins. A fusion layer similar to that produced by the fused outer lobes posterior to the mantle isthmus is not produced anteriorly, there being no fusion of the outer marginal lobes in the anterior embayment of the mantle. As with the splitting of the anterior region of the ligament, this absence of fusion of the outer lobes is a result of the effect of the tangential component on the mantle margins.

The structure of the ligament in *G. humanus* is similar to that described by Trueman (1949, 1950) for *Tellina tenuis* and *Mytilus edulis*. The fusion layer is probably comparable to Trueman's layer 1*a*. In *Glossus humanus*, as in *Mytilus*, this layer becomes split in the mid-line above the posterior region of the inner layer of the ligament. There can be no doubt that the periostracum in *G. humanus* is continuous over the external surface of the ligament. Observation was aided by the presence of the characteristic bristles which serve to differentiate the periostracum from the underlying layers. Indeed, the only region of the shell not covered by periostracum appears to be the 'outer' layer secreted by the mantle edge anterior to the isthmus. Over the posterior region of the ligament the periostracum is, however, often worn and only patches are left adhering to the outer surface.

Habit and Habitat

G. humanus has been described as an inhabitant of sand, sandy-mud and mud. The specimens from the Clyde Sea Area were found in very soft mud in company with such other typical mud-dwellers as *Brissopsis lyrifera*, *Nucula sulcata* and *Abra alba*. Occasional specimens of *Aporrhais serresiana*, a typical soft-mud dweller (Yonge, 1937) were also found. Colonies of *Perigonimus* sp. were often found growing on the shells of older specimens near the posterior margins of the valves. On one extremely large specimen obtained in an otter trawl from Loch Sween and measuring 11·0 cm long by 10·2 cm deep, there were a number of small anemones attached near the posterior margins of the valves.

The species is probably restricted to a soft muddy substratum since the inflated form of the shell and the relatively weak musculature of the foot would render it unfit for life in firmer substrates. *Glossus humanus* is a suspension feeder and in soft substrates there is danger of sinking beneath the surface with subsequent fouling of the short siphons. The light weight of the shell is probably associated with the habitat. It is interesting to compare *G. humanus* with the similar-sized bivalve *Arctica* (*Cyprina*) *islandica*, an inhabitant of coarser and firmer substrates of sand and sandy mud. In this species, the spiral angle is low and the shell much more compressed laterally. The shell is thick and heavy and the foot large and extremely powerful. Specimens of *Glossus humanus* and *Arctica islandica* were placed on sand in an aquarium tank. The specimens of *A. islandica* found little difficulty in burrowing rapidly into the sand until their siphons were flush with the surface. One specimen of *Glossus humanus*, after an interval of 12 hr., did succeed in covering approximately a third of the shell; the others remained more or less on the surface of the sand. The most critical feature restricting the animal to a soft type of substrate may well be the poor elastic properties of the hinge ligament. According to information received personally from E. R. Trueman, the opening thrust of the ligament of *Arctica islandica* is about eight times that of *Glossus humanus*. Occasional small specimens of *G. humanus* were found in the Clyde Sea Area in somewhat firmer muds than those off the coast of Arran but medium or large specimens were never obtained from these areas. This could be explained by the fact that the elastic properties of the ligament decrease with increase in size of the animal. In young specimens, cutting of the muscle attachments causes the valves to gape, but in older specimens the ligament appears to produce no opening thrust, the valves remaining closed when the muscles are cut.

Reports on vertical distribution are confused. Jeffreys (1876) records the fry and very young as ranging from depths of 50 to 1785 fathoms (91–3265 m.) in the North Atlantic and from 40 to 1456 fathoms (73–2663 m.) in the Mediterranean. Nicol (1951) points out that Jeffreys misidentified species of *Kelliella* for the young of *Glossus humanus* and that the latter probably range from 5 to 150 m. The specimens from the Clyde Sea Area were obtained from depths of more than 50 m.

The foot of *G. humanus* possesses a well-developed byssus gland and groove but threads were observed only in the younger specimens. The foot is capable of considerable extension (Fig. 5, F) but appears not to be much used in the larger forms. The siphons are short and surrounded by a common sheath of some ninety or more tentacles; the inhalant siphon is surrounded by a second inner sheath of approximately fifty tentacles. The siphons are flecked with reddish brown and opaque white areas, and their musculature appears to be more or less identical with those of *Aloidis* as described by Yonge (1946). The animal is extremely sensitive to any form of vibration. Specimens placed in a jar withdrew their siphons when a hand was placed lightly on the bench on which the jar stood. This sensitivity is further evidence that *Glossus humanus* normally inhabits quiet waters.

The Mantle

The mantle edge consists of the usual three lobes, the innermost being fused except in the regions of the pedal gape and the siphons (Fig. 5). The mantle is thin and delicate, the inner epithelium being ciliated and concerned with the elimination of waste material from the mantle cavity. The general trend of particles affected by this ciliation is from anterior to posterior toward the inhalant siphon. The main tract is from a region near the labial palps, ventrally and posteriorly to the region of fusion of the inner mantle lobes at the posterior end of the pedal gape (PG), and then posteriorly to the inhalant siphon (I). An interesting and apparently unique feature is that the pseudofaeces do not collect in a single mass at the base of the inhalant siphon but accumulate laterally on the side walls of the siphon (PS).

Mucus is supplied by glands distributed among the cells of the inner epithelial layer of the mantle. In addition, there are, in the connective tissue immediately beneath the epithelial layer, numerous spaces filled with a granular material. These spaces extend in a broad band dorsal to the ventral ciliated tract of the mantle from a region near the anterior adductor muscle to the posteriorly situated siphons. The contents are discharged on to the inner mantle surface through narrow pores extending between the epithelial cells. The nature of this substance is somewhat obscure. It gives a positive reaction with Schiff's leucofuchsin after oxidation with periodic acid. This indicates a polysaccharide component. Staining for glycogen with full controls gives negative results, as does the Toluidine Blue metachromatic reaction for chondroitin and mucoitin sulphuric acids. Alcian Blue 8 GS (Steedman, 1950)—a mucin stain—fails to stain it. Nevertheless, it seems probably to be some form of mucin-like substance, using the term mucin in a physical sense.

ORGANS IN THE MANTLE CAVITY

Both anterior and posterior adductor muscles are well developed, the anterior being about half the size of the posterior (Fig. 5). The pedal muscles are delicate with the anterior somewhat stouter than the posterior (PR). The labial palps are well developed and function in the usual manner (LP).

The ctenidia are heterorhabdic and deeply plicate with the inner demibranchs somewhat deeper than the outer (Fig. 5). There is an extensive supra-axial region (SA) and both inner (ID) and outer (OD) demibranchs curve inward over the visceral mass. Cuticular fusion of the type described by Atkins (1937*b*) connects the inner lamellae of the inner demibranchs of the two ctenidia, the inner lamellae of the inner demibranchs and the visceral mass, and the outer lamellae of the outer demibranchs and the mantle. Where the visceral mass passes between the two inner demibranchs posteriorly, there is a short region where the inner lamellae are not fused to the visceral mass or to one another. As in *Aloidis* (Yonge, 1946), a direct connexion can be established between the inhalant chamber below and the exhalant chamber above.

As shown in Fig. 5, material is carried by the frontal cilia to the free edges on both sides of the inner demibranch, on the outer surface of the outer demibranch, and on the ventral half of the inner surface of the outer demibranch. A narrow marginal groove is present at the free edges of both the inner and outer demibranchs. The frontal cilia of the dorsal region of the inner lamella of the outer demibranch convey material to the ctenidial axis where there is a strong anteriorly directed current. Material from the inner marginal groove passes directly between the palps to the mouth while material from the outer marginal groove and the axis reaches the mouth by way of the distal oral groove.

As noted by Ridewood (1903), the plications of the gill are not evenly spaced but are arranged in pairs. The inter-lamellar septa are alternately high and low so that, in the dorsal regions of the demibranchs, one principal filament is associated with an inter-lamellar septa while the next is not. Lateral cilia are well developed and produce a powerful inhalant current. Large eu-latero-frontal cilia, 35 μ in length, guard the inter-filamentar spaces, and there are smaller pro-latero-frontal cilia between them and the frontal cilia. The ciliation is similar on all filaments including the principal filaments.

Despite the deep plication of the gills, the walls of the marginal grooves are not scalloped since the plicae, as in *Solecurtus*, *Cardium* and *Lutraria* (Atkins, 1937*a*), tend to smooth out toward the free margins. Examination of the free margins of the gill under the microscope show what appear to be clear peg-like processes, some 10μ long, projecting from the filaments on either side of the marginal groove. Staining with Heidenhain's haematoxylin indicates that they are made up of tufts of cilia comparable to the 'sensory' tufts of cilia occurring on the frontal surfaces of the filaments of the gills in a number of species (Atkins, 1936). In *Glossus humanus*, they are largely restricted to the free margins of the posterior regions of the gills.

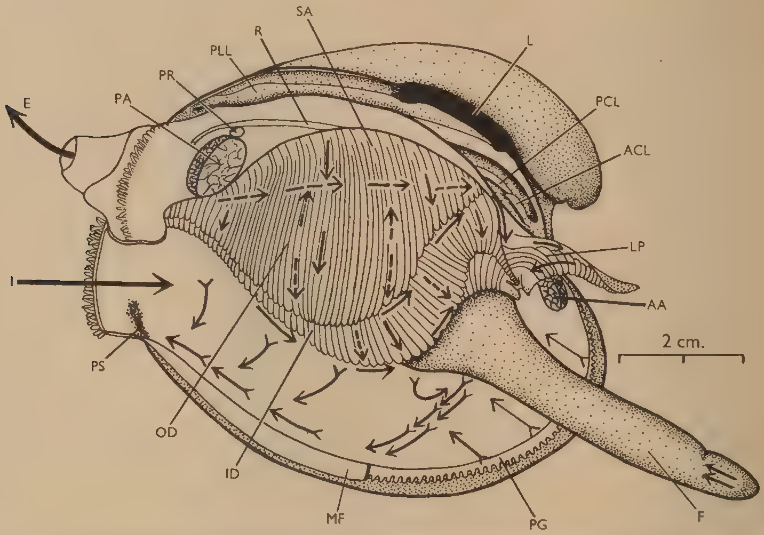

Fig. 5. *G. humanus*, organs of the mantle cavity exposed after removal of the right shell valve and mantle lobe. F, foot; ID, inner demibranch; LP, labial palp; MF, region of fusion of inner marginal lobes; OD, outer demibranch; PG, pedal gape; PLL, left posterior lateral tooth; PR, posterior retractor muscle; PS, pseudofaeces; R, rectum; SA, supra-axial region. Large arrows indicate inhalant (I) and exhalant (E) currents; small arrows, ciliary currents on exposed surfaces; broken arrows, currents on inner surfaces of demibranchs; feathered arrows, rejection currents. Other lettering as before.

Despite the muddy type of substrate, guard cilia (Atkins, 1937*a*) are not present at the edges of the marginal grooves. As in many lamellibranchs, coarse cirrus-like cilia, 40–50μ long, occur along the free margins of both the inner and outer demibranchs. Similar cilia occur scattered over the frontal surfaces of the filaments and on the surface of the ctenidial axis. Those present on the filaments attain a length of some 70μ while those of the axis reach the surprising length of 130μ. Presumably, as suggested by Yonge (1946), they are concerned with the movement of large particles and masses.

Atkins (1937*a*) has emphasized the part played by the gills of most lamellibranchs in the sorting of material. The narrow marginal grooves in *G. humanus* ensure that only small particles are conveyed anteriorly toward the palps and mouth. Large particles and masses fail to enter the marginal grooves and soon fall off the gill edge on to the rejectory tracts of the mantle surface. The paired arrangement of the plicae may also help in the rejection of material. Contraction of the gill musculature results in the apposition of adjacent plicae; presumably this would happen if the inhalant current contained a considerable amount of suspended matter. With the gill in this condition, particles removed from the water tend to concentrate on the almost flat portions of the lamellae between the pairs of closely approximated plicae; they travel ventrally toward the free edges. The presentation of these 'concentrated' masses of particles to the narrow marginal grooves increases the possibility of their rejection on to the ciliated surface of the mantle.

The gills of *G. humanus*, however, cannot be regarded as adapted for dealing with water containing large amounts of sediment. The deep plication may be considered primarily as a means of increasing the collecting surfaces of the ctenidia; it will also increase the power of the inhalant current. Thus, despite the muddy nature of the substrate to which the species is almost certainly restricted, the gills in *G. humanus* appear to be adapted for dealing with large volumes of water containing relatively small quantities of suspended material. Such conditions are likely to occur in water where there is little or no disturbance of the bottom deposits.

THE ALIMENTARY CANAL

The mouth, situated between the paired palps, leads into the oesophagus which is compressed dorso-ventrally and richly supplied with mucous glands. It enters the antero-dorsal region of the stomach, which lies in the dorsal region of the visceral mass and is enclosed antero-laterally and ventrally by the digestive diverticula. Leaving the stomach posteriorly and ventrally is the combined style sac and mid-gut, partially separated from one another by the major and minor typhlosoles. The style sac is curved and extends ventrally to the junction of the foot and visceral mass. Within the visceral mass, and anterior to the style sac, the mid-gut is thrown into three loops. The hind-gut ascends dorsally, posterior to the style sac, to penetrate the pericardium and pass through the ventricle of the heart. A well-developed aortic bulb extends posteriorly from the ventricle to the posterior adductor muscle. The hind-gut lies within this aortic bulb attached to the mid-dorsal wall. Finally, the hind-gut passes over the posterior adductor to open at the anus near the exhalant siphon.

The Morphology of the Stomach

The external form of the stomach in *G. humanus* is shown in Fig. 6. Dissections were carried out on living specimens and the course of the ciliary currents followed by means of fine carborundum powder and carmine. No matter how carefully the stomach was opened, some part of the internal anatomy was invariably lost or distorted. The drawing of the stomach is therefore the result of observations on a number of specimens, the stomachs having been opened by a variety of incisions (Fig. 7). As far as possible the nomenclature introduced by Graham (1949) has been used. It is proposed, however, as suggested by Purchon (1953), to describe the 'right half' and 'left half' of the caecum as the 'right caecum' and 'left caecum' respectively.

The stomach in *G. humanus* may be conveniently divided into three regions; a globular posterior region, an anterior receiving region, and a dorsal hood. The dorsal hood (Fig. 6, DH) extends anteriorly over the posterior part of the stomach and ends blindly on the left dorsal side as a small coiled tube (CDH). The greater part of the stomach consists of the posterior globular region (Fig. 7). Leaving this region ventrally is the combined style sac and mid-gut (SS). The minor typhlosole (T_2) ends at the junction of the style sac with the stomach while the major typhlosole (T_1) extends as a flap-like structure anteriorly across the floor of the stomach. As in the majority of Eulamellibranchia, it is continued into the right (ORC) and left (OLC) caeca (Graham, 1949) to form broad tongue-shaped structures (Fig. 6, EC). Continuous with the mid-gut and following the course of the major typhlosole is the intestinal groove (Fig. 7, GI). The posterior sorting area (SAP) is well developed, extending dorsally over the right wall of the stomach and on to the right wall of

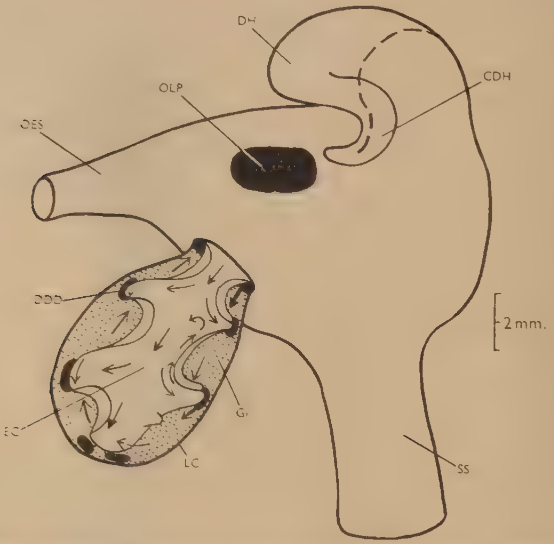

Fig. 6. *G. humanus*, diagram of the external appearance of the stomach. CDH, coiled region of dorsal hood; DDD, opening of duct from digestive diverticula; DH, dorsal hood; EC, extension of typhlosole within left caecum; GI, intestinal groove; LC, left caecum cut open; OES, oesophagus; OLP, opening of left pouch; SS, combined style sac and mid-gut. Arrows indicate ciliary currents within the left caecum.

the dorsal hood. The posterior margin of the sorting area is formed by a well-developed fold (PF) while the anterior margin is formed by the rejection groove (RG) which drains into the intestinal groove on the floor of the stomach. The anterior margin of the rejection groove is formed by a second fold bearing a number of small parallel ridges (AF).

The openings of the ducts to the digestive diverticula are to a considerable extent concentrated within the right and left caeca. The ducts of the right dorsal regions of the diverticula open independently on the right dorsal wall of the stomach (RD) while those of the left dorsal regions open into the left pouch. The pouch opens into the stomach by a single large aperture (OLP) near the gastric shield (GS). The gastric shield consists of a thickened convex portion between the apertures of the coiled region of the dorsal hood (OCH) and the left pouch (OLP) and a larger wing-like expansion extending over most of the left posterior wall of the stomach and the left ventral wall of the hood. A spur-like extension of the shield projects into each of the openings helping to maintain the shield firmly in position.

The anterior receiving region of the stomach is little more than an expansion of the oesophagus (OES). The entrance of the oesophagus into the stomach is marked by a circular groove (CG) which is continued posteriorly over the roof of the stomach (DG). This dorsal groove curves over the ventral lip of the hood, between the thickened portion of the gastric shield and the anterior fold, to terminate on the ventral floor of the hood (Fig. 8).

The posterior sorting area, together with the rejection groove and bounded by the anterior and posterior folds, extends on to the right wall of the dorsal hood (Fig. 8, SAP). The anterior fold curves round on to the antero-ventral face while the posterior fold forms a crest over the mid-dorsal line of the hood. Both anterior and posterior folds terminate within the coiled portion of the hood, the anterior in a curious circular protuberance. Extending over the roof of the hood to the left of the posterior fold is a powerfully ciliated acceptance tract which is continued ventrally over the posterior wall of the stomach (AT). Over the left wall of the hood and extending to the margin of the gastric shield is a series of poorly developed folds.

The Functioning of the Stomach

That in the Lamellibranchia both intracellular and extracellular digestion occur is generally agreed. The crystalline style is the source of certain extracellular enzymes which are liberated into the stomach by the dissolution of the style (Yonge, 1925). Intracellular digestion takes place within the cells of the digestive diverticula (Yonge, 1926*a*). With these facts it should be possible to estimate some of the more important mechanical requirements of the lamellibranch stomach. For the efficient utilization of the extracellular enzymes the ingested particles should be brought into intimate contact with the crystalline style. Secondly, for intracellular digestion, it is essential that fine particles be presented to the digestive diverticula. This may be achieved either by an effective sorting mechanism capable of selecting suitable particles or by the ingested particles undergoing a process of trituration. Finally, the waste materials from the digestive diverticula, consisting of solid excretory products and rejected particles, should be prevented from returning to the gastric cavity.

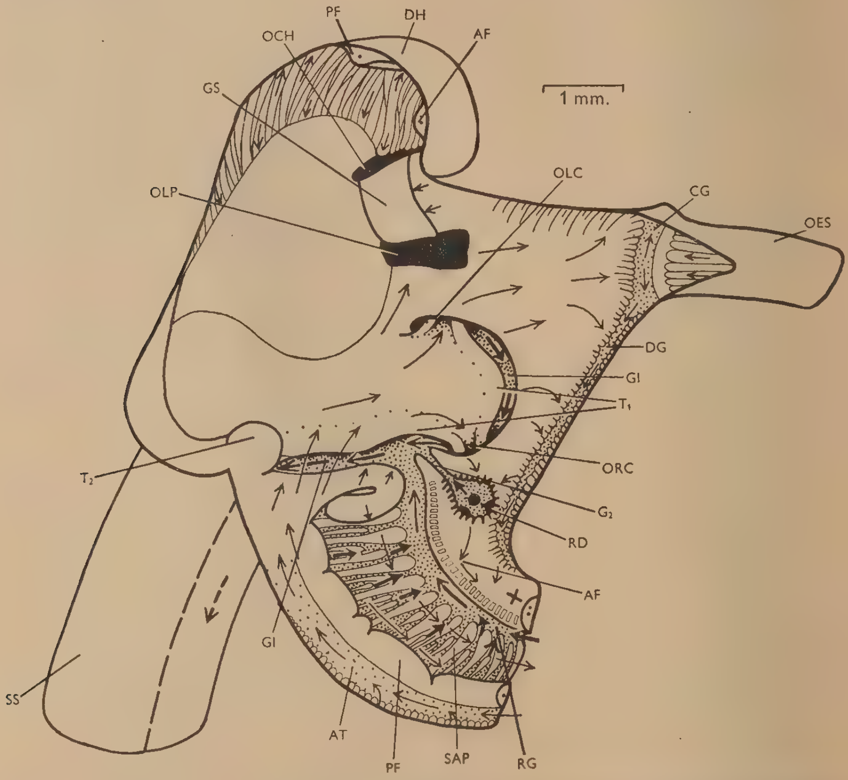

Fig. 7. *G. humanus*, stomach opened by a dorsal and posterior incision and the right wall deflected ventrally. AF, anterior fold; AT, acceptance tract; CG, circular groove; DG, dorsal groove; GS, gastric shield; G_2, rejection groove from isolated ducts of right side; OCH, opening of coiled region of dorsal hood; OLC, opening of left caecum; ORC, opening of right caecum; PF, posterior fold; RD, opening of isolated ducts of right side; RG, rejection groove; SAP, posterior sorting area; T_1, major typhlosole; T_2, minor typhlosole. Other lettering as before. The arrows indicate the direction of ciliary currents.

To understand the functioning of the stomach it is necessary to realize that the crystalline style rotates. Unfortunately the style has not been observed revolving in *G. humanus*, but investigation of the cilia of the style sac suggests that it revolves in a clockwise direction when viewed from above. This is supported by the observations of workers who have viewed the rotation of the style in other species; Nelson (1918) in *Modiolus* and *Anodonta*; Yonge (1926*b*) in the spat of *Ostrea edulis*; Yonge (1949) in *Tellina tenuis*; Yonge (1951*a*) in *Sphenia binghami*; Yonge (1951*b*) in *Cryptomya californica*. The statement by Yonge (1951*b*) that the style in *Cryptomya* rotated in an anti-clockwise direction when viewed from the anterior end was printed in error (personal communication).

In *Glossus humanus* particles entering the stomach from the oesophagus are conveyed dorsally by the cilia of the circular groove and then directed posteriorly by the cilia of the dorsal groove (Fig. 7). At the ventral lip of the dorsal hood (the region marked X) the particles are directed against the ridges of the anterior fold (AF). Here, at least when the stomach has been opened for observation, the particles embedded in mucus collect and remain stationary. In the intact stomach the revolving crystalline style may reasonably be presumed to brush these particles on to the ridged surface of the posterior sorting area (SAP). The ciliary mechanisms of the grooves and ridges of this area exercise a quantitative selection. The heavier particles are carried by the cilia of the grooves to the rejection groove (RG) and so to the mid-gut by way of the intestinal groove (GI). The lighter particles are carried dorsally, being thrown from crest to crest across the ridges. Thus, only the finest particles are retained and reach the distal regions of the dorsal hood (Fig. 8). Within the dorsal hood they are directed to the edge of the gastric shield and included in the revolving mass at the head of the crystalline style. The style is relatively delicate and almost certainly incapable of causing any effective trituration of particles.

The acceptance tract (AT) conveys particles from the head of the crystalline style over the roof of the dorsal hood and ventrally over the posterior wall of the stomach (Fig. 7). Where this current has been described in previous accounts it has generally been considered as supplying particles to the mid-gut. In *G. humanus*, however, this current serves to carry particles on to the floor of the stomach. The entrance of particles into the mid-gut is prevented by the flap-like structure of the major typhlosole which extends over the intestinal groove. The currents on the floor of the stomach are directed towards the openings of the left pouch (OLP), the left (OLC) and right (ORC) caeca and the isolated ducts of the right side (RD). Within the right and left caeca the ciliary currents over the extensions of the major typhlosole direct particles towards the openings of the ducts into the digestive diverticula (Fig. 6, DDD).

The material carried by the acceptance tract should therefore consist of fine particles which have previously been in contact with the crystalline style. The pattern of ciliary activity within the dorsal hood is shown in Fig. 8. It was at first thought that the poorly developed folds over the left wall of the hood might constitute a sorting area. Detailed investigation of the ciliary currents did not reveal any selective action. The currents over the ventral regions of the folds direct particles to the edge of the gastric shield while those of the dorsal region direct particles to the acceptance tract. An understanding of the function of these currents can obviously only be obtained through an appreciation of their dynamic relationships with the revolving crystalline style. Fig. 9 shows a simplified diagrammatic representation of the circulation of material within the stomach. The material entering from the oesophagus (OES) consists of a mixture of fine and coarse particles. These particles are caught up by the revolving crystalline style (CS) and brushed on to the posterior sorting area. The ciliary currents of the sorting area direct the coarse particles to the mid-gut (MG) by way of the rejection groove (RG) and the intestinal groove (GI). The fine particles are conveyed dorsally. Thus the material being revolved by the crystalline style consists of fine particles at the head of the style passing into a mixture of fine and coarse particles below. The ciliary currents over the dorsal regions of the folds within the dorsal hood therefore convey fine particles from the

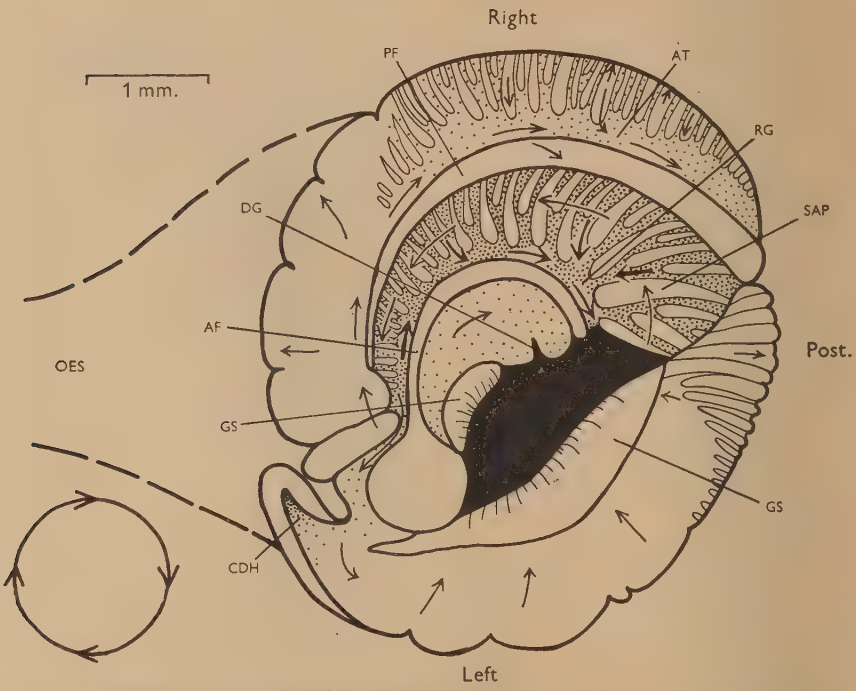

Fig. 8. *G. humanus*, dorsal hood of stomach opened along the broken line shown in Fig. 6 and viewed dorsally. Lettering as before. Arrows indicate the direction of ciliary currents; the circular arrow indicates the clockwise direction of rotation of the crystalline style when viewed dorsally.

head of the style to the acceptance tract (AT). The currents over the ventral regions of the folds act on a mixture of fine and coarse particles directing them to the edge of the gastric shield and so returning them to the mass around the crystalline style. Some coarse particles may be caught up in the ciliary currents of the acceptance tract as it passes ventrally over the posterior wall of the stomach, although the tract is 'protected' to some extent by the presence of the posterior fold along the right-hand margin. Particles too large to enter the openings of the left pouch, the right and left caeca, and the isolated ducts of the right side are carried anteriorly to join the incoming material and so circulate again. Within the right and left caeca waste products and rejected particles from the ducts of the diverticula are caught up by the ciliary currents in the intestinal groove (GI) and conveyed to the mid-gut. Rejected material from the isolated ducts of the right side is conveyed to the intestinal groove by way of the short groove G_2 (Fig. 7). The ducts of the diverticula which open within the left pouch return material to the general circulation within the stomach.

Most of the mechanical requirements mentioned earlier are fulfilled in the stomach of *G. humanus*. The ingested material is subjected to a rigorous sorting which appears to be entirely quantitative and the 'accepted' particles are brought into intimate contact with the style and so with the enzymes released from it. The flap-like major typhlosole isolates the currents in the intestinal groove. This agrees with the description by Graham (1949) of the intestinal groove as the 'private pathway of the digestive gland and sorting areas'. In the Eulamellibranchia there appears to be a progressive concentration of the ducts of the diverticula within the right and left caeca. This isolation of the ducts within the caeca may be associated with preventing material rejected by the diverticula from returning to the stomach. Even so, in the majority of the eulamellibranchs, the ducts opening into the left pouch return their waste products to the general circulation within the stomach. This retention of an isolated opening for the left pouch may well be correlated with the need for a firm fixation of the gastric shield. In most species the shield is anchored firmly by the spurs which project into the openings of the left pouch and the coiled region of the dorsal hood.

The stomach in *G. humanus* is well adapted for dealing rapidly with fine particles and is comparable with the stomachs of the suspension-feeders, *Paphia pullastra*, *Cardium edule* and *Venus fasciata*, as described by Graham (1949). Yonge (1949) has already discussed the reasons for not including *Tellina crassa* and *Solecurtus chamosolen* with the above. In both these species of the Tellinacea very coarse particles are taken into the stomach and the style acts as an efficient organ of trituration. Here the possession of an extensive sorting area would be a disadvantage. In fact, in *Tellina* and *Solecurtus*, the grooves and ridges of this region are very poorly developed or even absent.

The efficiency of the stomach mechanism may be judged by a consideration of the paths by which particles are able to enter the mid-gut. The material carried by the intestinal groove is derived from the rejection groove of the posterior sorting area and from the rejectory currents of the digestive diverticula. The rejection groove eliminates those particles rejected by the sorting area. All other particles remain within the gastric cavity until they have been 'presented' either to the ducts of the digestive diverticula within the caeca or to the isolated ducts of the right side. This retention of particles within the stomach is possible because the currents in the intestinal groove are isolated by the flap-like major typhlosole.

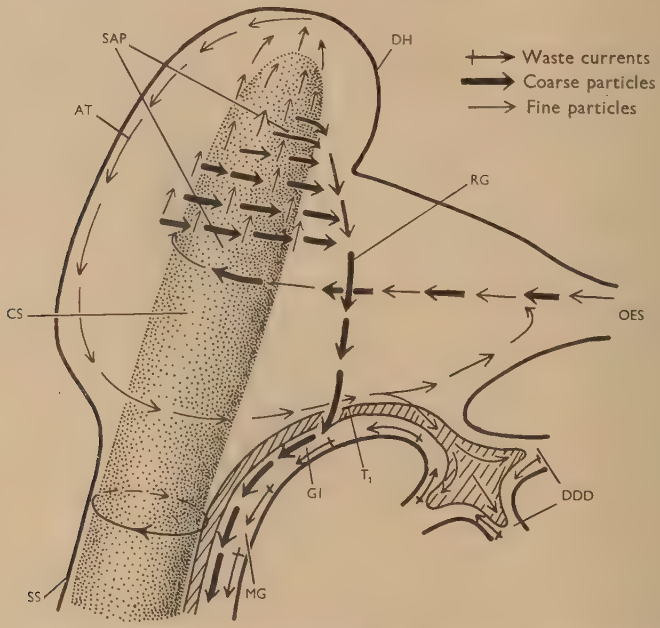

Fig. 9. *G. humanus*, diagrammatic representation of the circulation of particles within the stomach. CS, crystalline style; MG, mid-gut; SS, style sac. Other lettering as before. The heavy arrows indicate coarse particles, the light arrows fine particles, and the tailed arrows particles rejected by the ducts from the digestive diverticula.

Reproduction

Glossus humanus is unisexual and both the testes and ovaries are white when ripe. The gonads ripen towards the end of August and during September. Artificial fertilization was attempted on 3 September with some success, a small number of apparently normal trochophore larvae being obtained.

There was little doubt, however, that at this time the ovaries were not fully ripe. Thus, as in *Aloidis* and *Cuspidaria* (Yonge, 1946), spawning appears to occur at the end of September. Yonge has suggested that this autumnal spawning may indicate origin in deep water where the highest temperatures occur late in the season.

DISCUSSION

Glossus humanus is specialized for life in a substratum of very soft mud. The poor elastic properties of the ligament, the inflated form of the shell, and the relatively weak musculature, all suggest a sedentary mode of life within a soft substratum. The siphons are extremely sensitive, and it is unlikely that large quantities of material are normally taken into the pallial cavity. The marginal grooves of the gills are very narrow and convey only fine particles to the labial palps and mouth. These adaptations indicate that *G. humanus* is normally an inhabitant of fairly deep water where there is little or no disturbance of the bottom deposits. Under such conditions the surrounding water will contain little suspended material. If the animal is to obtain sufficient food large volumes of water must be passed through the gills. Their well-developed plications must be considered primarily as an adaptation for dealing with large volumes of water containing little suspended material. This restriction of the species to deep water is further supported by the late autumnal spawning.

In *G. humanus* the mechanical functions of the stomach appear to be carried out by ciliary activity aided by the revolving crystalline style. In other Eulamellibranchia, however, the entry of particles into the ducts to the digestive diverticula appears to take place by some means other than cilia (Purchon, 1953). This may also occur to a lesser extent, in *G. humanus*. The part played by muscular activity in the functioning of the eulamellibranch stomach has, on the whole, been neglected. Patterson (1933) introduced a rubber balloon into the stomach of *Schizothoerus nuttallii* and so recorded pressure changes there. Slight stomach contractions occurred at the rate of one per minute regardless of the presence or absence of the crystalline style. While the experiment needs to be repeated, it is certainly true that slight changes in pressure within the stomach would aid the entry of particles into the ducts of the diverticula. The particles affected by such small changes in pressure would be those carried anteriorly over the floor of the stomach past the openings of the right and left caeca and the left pouch. These would be the fine particles from the head of the crystalline style which are carried ventrally over the posterior wall of the stomach by the acceptance tract.

Recently there has been much discussion regarding digestion in the Lamellibranchia. Mansour & Zaki (1947) claimed that lipolytic and proteolytic enzymes are secreted into the lumen of the stomach by the digestive diverticula. They suggested that the cells of the diverticula fragment and pass, together with their contained enzymes, into the stomach. George (1952), investigating the digestion of fat in lamellibranchs, found that free droplets of neutral fat were hydrolysed in the stomach. Tests on the lipolytic activity of minced styles of several species all gave positive results, while only some species gave positive results in tests on minced digestive diverticula. Nevertheless, it is difficult to associate complete extracellular digestion with the organization of the stomach found in *Glossus humanus*. Graham (1949) discussed the functioning of the gastropod stomach and indicated the evolutionary trends associated with increased extracellular digestion and the secretory activity of the digestive gland in members of this class. In carnivorous species, such as members of the Stenoglossa, the stomach is little more than a bag where digestion takes place. There is no necessity for a separation of particles on a size basis and sorting areas are not present. The same conditions prevail in many of the pulmonates and opisthobranchs in which extracellular digestion predominates. This directly contrasts with conditions in the Eulamellibranchia, as exemplified by *G. humanus*, where there is an efficient sorting mechanism selecting particles on the basis of size.

The ciliary currents of the intestinal groove convey to the mid-gut particles discharged from the ducts of the diverticula within the right and left caeca. The flap-like major typhlosole prevents material carried by the intestinal groove from returning to the general circulation within the stomach. In contrast to Mansour & Zaki (1947), Coe (1947) suggested that the fragmented cells and minute brownish globules passed into the digestive canal from the diverticula were excretory. Morton (1951), investigating the gastropod family Struthiolariidae, came to the same conclusion. In members of this family fragmentation of the digestive cells results in tiny boluses of excretory matter containing nuclei, phagocytic cells and brown excretory spherules being passed into the gut. Morton emphasized that, although small traces of enzyme were doubtless liberated in this way into the stomach, the fragmented particles were essentially excreta.

Knowledge regarding digestion in the Lamellibranchia is confusing. There seems to be little doubt that extracellular lipases and proteases are present in the gastric cavity, although their source is uncertain. At the same time the complex ciliary mechanisms suggest a considerable degree of intracellular digestion as postulated by Yonge (1926*a*).

SUMMARY

Glossus humanus (L.) (*Isocardia cor* Lam.), a large marine eulamellibranch, occurs in deep water in soft muddy substrates. Restriction of the species to a soft substrate is probably correlated with the inflated form of the shell, the weak musculature of the foot, and the poor elastic properties of the ligament.

Growth of the mantle/shell is the resultant of radial, transverse and tangential components, the normal axis being a turbinate spiral. The anterior

region of the ligament is progressively split during growth owing to the displacement posteriorly of the secreting surfaces. The relationships of the valves, ligament and mantle isthmus are discussed.

The deeply plicated ctenidia are specialized for dealing with large volumes of water containing little suspended material.

The anatomy and functioning of the stomach is described in detail. There is a complex ciliated mechanism selecting particles on the basis of size. Such a mechanism, it is suggested, must be associated with intracellular digestion. The flap-like major typhlosole prevents material carried by the intestinal groove from returning to the circulation within the stomach, while the retention of an isolated opening for the left pouch is probably correlated with the need for firm fixation of the gastric shield.

Spawning occurs at the end of September.

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THE GROWTH OF *BALANUS BALANOIDES* (L.) AND *B. CRENATUS* BRUG. UNDER VARYING CONDITIONS OF SUBMERSION

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(Text-figs. 1–7)

There is some information in the literature on the growth rate of *Balanus balanoides* (L.) under varying natural conditions, but very few comparable data on *B. crenatus* Brug. exist. Most of this work relates to natural populations where crowding and other factors make accurate measurement difficult. In general, samples of a population, sometimes of unknown or only estimated age-distribution, have been measured at intervals. In the work reported below, the growth of numerous individual barnacles of known age, exposed to both intertidal conditions and complete submersion, has been followed through several successive seasons at Millport. In addition, some observations are presented on the effect of tidal level on the intensity of settlement and subsequent mortality of *B. balanoides*.

The Experimental Methods

For the intertidal exposures (*B. balanoides* only), seven 15 × 10 in., rough, black, plastic panels were bolted on to a long metal frame which was lashed in a fixed vertical position to the outer face of a pier pile, the position of each panel in relation to Chart Datum being calculated from known Ordnance Datum. The level of the centre of each panel is shown in Table I.

Observations during periods of calm weather at the site have shown that, in general, there is a closer agreement between the heights actually reached and those predicted for Greenock from new data in the *Admiralty Tide Tables for 1952* (1951), than with the values obtained by applying the corrections given for Millport. Mean tidal levels at the site, relative to Chart Datum, are therefore similar to those for Greenock, which are as follows: M.H.W.S., +11·1 ft.; M.H.W.N., +9·3 ft.; M.T.L., +6·2 ft.; M.L.W.N., +3·2 ft.; M.L.W.S., +1·1 ft. The hours of exposure and submergence at the centre of each panel over the actual period of settlement (also shown in Table I), and over a typical full lunar period (data for Figure 4), were calculated from graphs of the tidal cycle drawn up from data for Greenock in the *Admiralty Tide Tables for 1951* (1950), with a correction based on the new mean levels given in the 1952 tables. Anemometer records near the site show that wind speeds during the period of settlement were low and therefore the effect of wind on tidal levels has been neglected.

For the permanently submerged settlement (including both *B. balanoides* and *B. crenatus*), plain and ground glass slides of several sizes were exposed in a vertical position at a constant depth of 3 ft. from a nearby raft, a description of which has been given by Pyefinch (1948).

TABLE I. SETTLEMENT OF *BALANUS BALANOIDES* ON INTERTIDAL PANELS 6–26 APRIL 1951

The levels refer to the centres of panels and are given in feet relative to Chart Datum; the number of cyprids refer to the upper, middle and lower thirds of each panel, together with total numbers; the tides across are the number of times the water surface passed the centre of the panel each way during the settling period; exposure and submergence in hours and percentage of total (see text).

Panel No.	Level	Cyprids		Tides across	Exposure	Submergence
I	+10·8	0	0	34	460	30 (6 %)
II	+8·9	50 154 159	363	76	368	122 (25 %)
III	+7·2	1077 670 565	2312	80	283	207 (42 %)
IV	+5·4	2509 1077 564	4150	80	212	278 (57 %)
V	+3·6	425 254 38	717	74	132	358 (73 %)
VI	+1·8	1837 555 148	2540	46	47	443 (90 %)
VII	0·0	806 174 169	1149	0	0	490 (100 %)

On all panels, after a quantity of spat had settled, the positions of a number (20–40 per set) of selected barnacles, uniformly distributed over the panels, were marked and these barnacles were thereafter measured at intervals. When in the laboratory for measurements, the panels and slides were kept in running sea water except while actually being measured and, with very few exceptions, were returned to the exposure sites within 24 hr. At each inspection the panels and slides, and with the older barnacles the surfaces of the barnacles themselves, were cleaned of debris, algae and settled animals.

Three dimensions were measured: the rostro-carinal axis (termed length), the greatest diameter at right angles to the length (termed breadth), and the perpendicular height of the tallest compartment (usually the carina) above the basis. The third dimension was measured on only a limited number of inspections, but the first two were measured throughout the observations. In the early stages of growth on both the large intertidal panels and the small submerged slides the lengths and breadths of the barnacles were measured by means of a binocular microscope with low-power objective and an eyepiece micrometer. In the later stages on the submerged slides a vernier-measuring microscope was used. Heights of the submerged barnacles were measured throughout with a micrometer screw-gauge, allowance being made for the thickness of the slide. The use of small slides greatly facilitated accurate measurement of the submerged barnacles, but led in later stages of growth to the necessary sacrifice of some individuals which had to be removed to prevent overcrowding and consequent distortion of growth. With the intertidal barnacles, heights, and at the later inspections length and breadth also, had to be measured as accurately as possible with a pair of fine pointed dividers, because of the large size of the panels.

The observations on the submerged barnacles extended continuously from April 1950, and on the intertidal barnacles from April 1951, to October 1952, readings being taken monthly during periods of rapid growth.

In most studies of barnacles increase in length of the rostro-carinal axis has been used as a measure of growth (see p. 120). Moore (1934), however, measured length, breadth and height and computed the volume of an equivalent cone of basal diameter equal to the mean of the length and breadth, and with the measured height. As Moore points out, the increase in size of the whole animal is obviously better measured by volume changes, but since, as will be shown, the shape does not change greatly with age, for many purposes the length may be conveniently used. However, the fact that length has been used must be borne in mind; the volume will approximate to a function of the cube of the length. The advantages of using the length are that it does not involve any assumptions about length/volume relationship, and that the present results are more readily compared with those of other workers. Further, it is a convenient method for field work since the length can be measured quickly and with reasonable accuracy.

Settlement and Survival on the Intertidal Panels

In 1951 the first settlement of *B. balanoides* was observed on the pier piles and on the shore on 10 April, and settlement continued during the remainder of this month. The panels were exposed throughout this period, during which several inspections were made. At none of the inspections were any cyprids found on the topmost panel (Panel I, level + 10·8 ft.), although settlements took place on all of the remaining panels (II–VII). Before marking the selected barnacles on 27 April the distribution of *all* the cyprids and young barnacles on each panel was ascertained. In Table I (p. 108) the distributions (dividing the panels into upper, middle and lower thirds) are shown. With the exception of Panel V (level + 3·6 ft.), the total settlement is much less on Panel II than on the remainder; that on Panel V is apparently anomalous, and the only explanation that can be put forward is that it is the result of different local conditions in the immediate vicinity of this panel brought about by a diagonal pier beam immediately behind the frame.

The numbers actually counted on any panel will represent the net result of cyprid settlement and mortality. It might be thought that cyprid settlement would increase with total submergence, and that the number of cyprids settling would be greater as the depth of panels increased. This is clearly at variance with the results (see Table I), and it seems difficult to find any neutralizing effect by which mortality could produce the observed net results in relation to panel depth. Exposure (to air), which might be expected to be the major factor responsible for cyprid mortality, since it is inversely related to submergence, clearly cannot produce the observed net result. The following theory is put forward to explain the observed distribution. Although it is known that *B. balanoides* will settle on a permanently submerged surface (indeed, quite good settlements are recorded here), under natural conditions it is usually an intertidal species and undoubtedly the greatest settlement takes place within the littoral zone. It is postulated that intertidal settlement takes place most readily from a thin layer of water, such as is normally obtained when water moves forwards and backwards over a solid surface. On vertically exposed panels this occurs while the tide is rising and falling past the panel. During this process the surface of the panel is continually being swept by films of water that drain away. The 'clinging reaction' of *B. balanoides* cyprids described by Pyefinch (1948) (in contrast to *B. crenatus*, which is mainly a sublittoral species) lends support to this hypothesis. On this basis, the important factor for settlement is the number of times the tide *crosses* the surface of the panel; this will be maximal in the centre of the tidal range, and less at the upper and lower levels (see Table I), and would lead to maximal settlement in the mid-tidal region. The major factor opposed to that producing settlement is considered to be mortality of cyprids caused by exposure to air, and the hours of exposure at the various levels during the period under consideration are shown in Table I. The low numbers on Panel II are therefore largely the result of high mortality consequent upon the very considerable exposure to air. The differences on Panels III and IV are due to the decreasing exposure factor, and the lower values on Panels VI and VII are due largely to the less frequent crossing of the panel by the tide, since the exposure factor and consequent mortality is here greatly reduced. The actual relation between exposure to air and mortality over the settling period is not quantitatively known, but mortality of cyprids and young barnacles is known to have been very high on Panel II. However, it is quite clear from the figures in Table I that the opposition of these two factors can produce a maximal net settlement about the level of mid-tide, in accordance with the observed results. A similar distribution of the settlements of *B. balanoides* over the littoral zone has been observed by other workers (see, for example, Hatton & Fischer-Piette, 1932; Moore, 1935*b*; Hatton, 1938). An extension of this hypothesis would also seem capable of explaining in part the well-known fact that *B. balanoides* tends to settle in greater numbers in situations with considerable exposure to wave action. In such places, increased surge and breaking waves will repeatedly spread a thin layer of water over a larger area than in sheltered situations.

The complete lack of survival on Panel I must be due to the extremely high exposure factor; but at this level on the adjacent pier pile, which is just above the upper limit of *B. balanoides* in quantity (10·2 ft.), a very slight permanent new settlement was observed, and this is ascribed to the rougher surface texture of the wooden piles. Such a surface affords a better key for settlement (see Barnes, Crisp & Powell, 1951), and also is less easily desiccated (see Pomerat & Reiner, 1942; Pomerat & Weiss, 1946; Barnes & Crisp, 1953).

A further noticeable feature of the settlement, with the exception of the high-level Panel II, is the distinctly greater settlement on the upper third of each panel (see Table I). This can readily be explained in terms of a light response, since it has been shown (Barnes *et al.* 1951) that the crawling cyprids react positively to light, and this accumulation could therefore be the result of the general movement of the cyprids up the vertical panels. Further, the accumulation is most marked on the lower panels, and perhaps may be related to the total submersion time, longer periods of submersion allowing more time for migration.

Mortality of Marked Barnacles

Of the forty-seven marked barnacles uniformly distributed on Panel II (situated at about M.H.W.N. level) all except ten were dead after 1 month's exposure. Only four survived 2 months' exposure and these continued to grow slowly (see below) until late summer. They were all absent by October and there was no evidence of any accident or predation; so that, although this is below the level at which barnacles occur in quantity on the adjacent pier piles, conditions on the smooth panel during the higher summer air temperatures were apparently too severe for survival.

On the remaining panels all the marked barnacles, except those deliberately removed to prevent overcrowding, survived for at least 1 year. It should be remembered that all panels were kept free of such predators as *Nucella lapillus* (L.) and *Asterias rubens* L. However, in July of the second season many were dead, probably as a result of infection by the parasitic isopod *Hemioniscus balani* (Spence Bate). The resultant mortality was 56% on the uppermost panel at this time (Panel III) and 20% on the lower panels (IV–VII), and these figures indicate that this parasitism may here not only cause considerable mortality of adult barnacles, but also exert a differential effect with respect to tidal level. Prenant (1923) records that approximately 20% (which he regards as a minimum) of *Balanus balanoides* growing on rock masses near Roscoff were parasitized by *Hemioniscus balani*.

SEASONAL GROWTH AND SPECIFIC GROWTH RATES

Balanus crenatus *on Submerged Panels*

Pyefinch (1948) has pointed out that, although the major liberation of nauplii and subsequent settlement of cyprids takes place in the spring in this area, ripe larvae are present in a proportion of the adults (although in diminishing numbers) throughout the summer; periodic outbursts of cyprid larvae are found in the plankton throughout the summer and early autumn. In 1950, when the present observations were begun, quite considerable settlements took place during the late summer and autumn—as late as October.

In all, the growth of seven distinct settlements was followed throughout three successive seasons. For comparison of growth each settlement (Series I–VII) has been considered separately, and the mean length and standard error calculated for each Series at each date of observation; the mean lengths are plotted against age in Fig. 1.

It is clear from the figure that rapid growth occurs after metamorphosis, and increase in length continues during the spring and summer months. In the animals settling from mid-April to mid-June (Series I–IV, termed Group I) growth continued until mid-September, by which time the mean length of the earliest settlers (Series I) had reached 21·7 mm. and this was only a few millimetres less than the maximum mean length attained over three seasons; the later Series IV of this Group had reached a mean length of 17·7 mm. by mid-September of the first season. In none of this Group was any significant growth recorded after mid-September, that is during the first autumn and winter. The second season's growth did not begin until the latter half of May 1951, a month later than the time at which rapid growth began in the previous season. In the second season the same general growth pattern was repeated in Group I but with greatly reduced intensity, growth taking place during the summer and ceasing in the autumn. The mean increase in length, however, was very small during this second season, not exceeding 4–5 mm.: clearly Group I individuals had almost reached their maximum size in the first season's growth.

Series V–VII (Group II) settled during the summer and autumn of 1950. Growth followed settlement as with the spring spat but, in marked contrast, increase in length continued much longer, some being recorded in late autumn and winter (October to December). Indeed Group I had virtually ceased to grow before the settlements of Series VI and VII occurred. Further, in sharp contrast to Group I, the latest settled individuals (Series VII) were showing some growth between January and February of the following season, more growth from February to March and rapid growth, comparable with the initial growth of the earliest settlements of the previous year, from March to April. Growth continued until early autumn of the second year, when the barnacles appeared to have reached their maximum size, which was com-

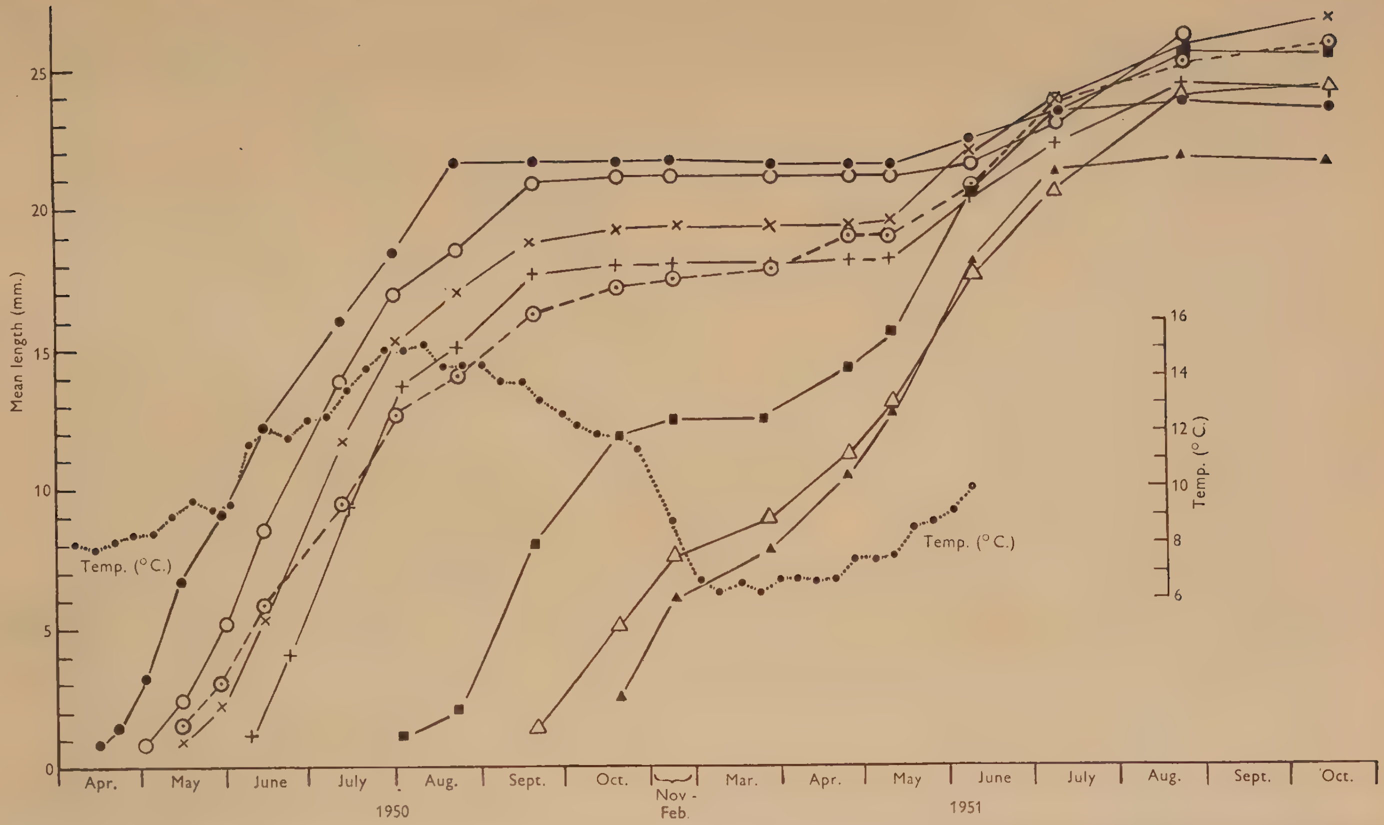

Fig. 1. Growth of *Balanus crenatus* and of *B. balanoides* over several seasons under conditions of total submersion. The mean lengths of each settlement (Series I–VII) at each date of inspection are shown: the almost negligible growth in the third season is omitted. The weekly mean sea-water temperatures (° C.) are also shown. *B. crenatus*: Series I, ●; II, ○; III, ×; IV, +; V, ■; VI, △; VII, ▲; *B. balanoides*, ⊙.

parable with that of Group I. No significant growth took place during the second winter. No appreciable growth (< 1·0–2·0 mm.) occurred in either Group during the third season.

The above account of the seasonal growth has been based on increases in length, and a similar picture emerges if the breadth is used. However, in order to compare the relative growth at different times, the size of the animal at the particular time under consideration must be taken into account. The specific growth-rate is used for such comparisons. The increase in length per unit length per day has therefore been calculated for each individual and the mean value obtained for each interval for all Series. (If multiplied by 100, for convenience, the values represent percentage increase in length per day.) All the values, except for those periods when there was no increase in length, plotted against the mean length during the interval concerned, are shown in Fig. 2. The intervals are not always exactly the same. The mean specific growth-rate diminished with increasing size. The points for Group I all fall on a smooth curve, indicating that the mean specific growth-rate over this period is a function of the size and is independent of the time of settlement within the group, since in these plots the ages are not considered. Food supply, temperature and other environmental factors which varied throughout the season appear not to have been overriding factors. However, the values of the mean specific growth-rate of Group II late in the season are less than

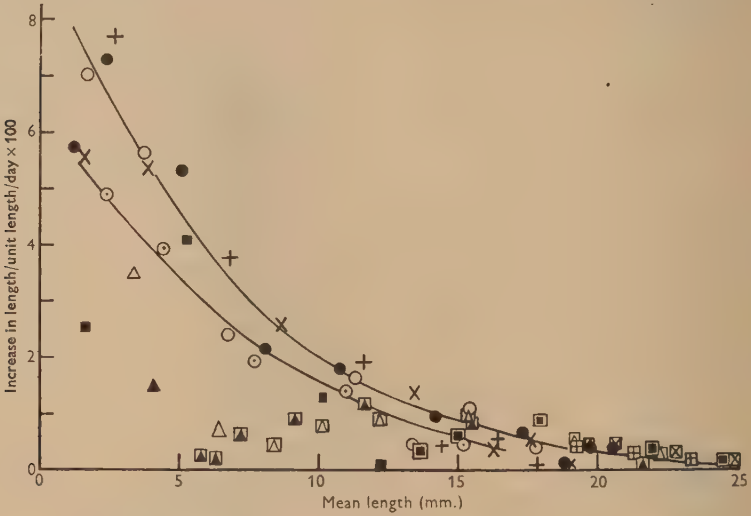

Fig. 2. The mean specific growth-rate (i.e. increase in length per unit length per day) × 100, plotted against the mean length, for all settlements of *B. crenatus* and a single settlement of *B. balanoides* under conditions of constant submersion. Symbols as in Fig. 1; those enclosed in squares are records for second season.

the corresponding values for Group I at the same length in the spring and early summer, suggesting that environmental factors were effective in limiting growth late in the season (see p. 124). Further, in the second season the mean specific growth-rates of Group II in the very early spring are less than those of Group I at the same size, growing rather later in the previous season. Indeed, during the latter part of the spring of the second season's growth, the mean specific growth-rate of Group II actually rises with an increase in mean length.

Balanus balanoides *on Intertidal and Submerged Panels*

In contrast to *B. crenatus*, there is only one liberation of nauplii during the year, and the settlement of cyprids usually takes place over the latter part of March and throughout April. There is therefore nothing comparable with the seven Series of *B. crenatus*, but only observations on the growth of a single settlement for each panel at different tidal levels. The mean lengths plotted against age are shown in Figs. 1 and 3. It should be remembered that growth of the intertidal barnacles was measured in the season following the observations on the submerged barnacles. The growth pattern is similar to that just discussed for *B. crenatus*. Increase in length takes place throughout the spring and summer months, but in contrast, some growth, although very small, appears to continue throughout the first winter on the intertidal panels, this continued growth being more marked on the upper panels where the

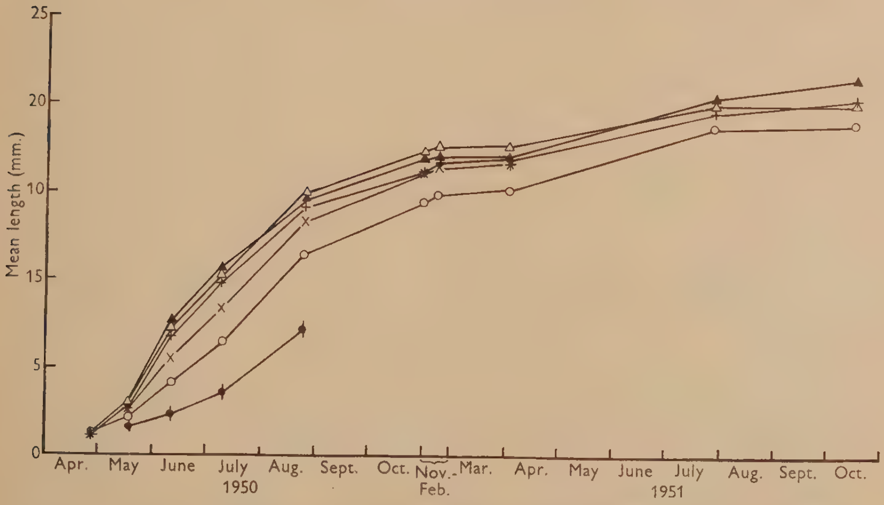

Fig. 3. Growth of *B. balanoides* over two seasons on intertidal panels II–VII (see Table I). The mean lengths on each panel at each date of inspection are shown. Panel II, ♦; III, ○; IV, ×; V, +; VI, △; VII, ▲.

barnacles were smaller. In the second season growth continued on all panels. By October of the first season the mean length attained by *B. balanoides* on the submerged exposures was 17·2 mm., and on the intertidal panels the mean length varied from 17·3 mm. on the lower Panel VII to 14·4 mm. on the upper Panel III (no barnacles remaining on Panel II at this date). Complete submersion has thus given optimum conditions for growth, and the growth on the lower intertidal panels closely approaches that for complete submersion. This is clearly brought out in Fig. 4 in which the mean length attained by 24 August 1951 (i.e. after approximately 4 months' growth) is plotted against the hours of submersion during one typical full lunar period.

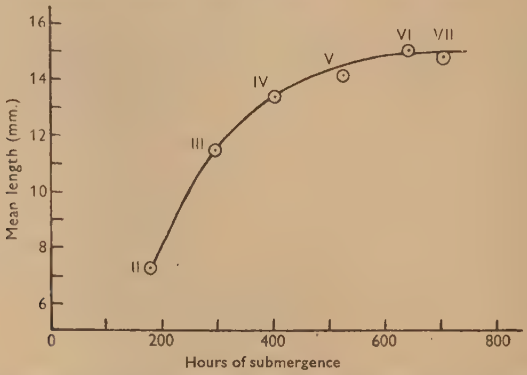

Fig. 4. The mean length attained by *B. balanoides* on intertidal panels, plotted against hours of submergence over a typical lunar cycle. The heights of the Panels, in feet (relative to Chart Datum) are as follows: II, 8·9; III, 7·2; IV, 5·4; V, 3·6; VI, 1·8; VII, 0·0.

The mean specific growth-rates for the barnacles on each panel over the first growing season are shown in Fig. 5. With the exception of the high level Panel II for which there are only three readings, the mean specific growth-rates fall throughout life. During the early stages of growth the mean specific growth-rate is very markedly influenced by the exposure. For example, on Panel II at a mean size of 2·0 mm. the specific growth-rate was 0·0141, while on Panel VII at 2·17 mm. it was 0·0387, and on the totally submerged panels at a mean length of 2·37 mm. it was 0·0489. The other intertidal panels give values intermediate between these extremes. At the levels of Panels V–VII there is little evident distinction between the growth-rates, although the points lie below those for complete submersion (raft) derived during the previous year. The mean specific growth-rates, however, fall more steeply on those panels with greater submersion, so that by the time a size of about 9 mm. is reached the mean specific growth-rate and the change of mean specific growth-rate with increasing length have become virtually identical, and at a slightly increased length are coincident with the values for complete submersion.

Apparently *B. balanoides*, unlike *B. crenatus*, does not reach its virtual maximum size in a single season, and therefore it is not surprising to find that the length began to increase significantly quite early in the second season and that relatively more growth took place during the second season, even though this was small in comparison with the first season's growth. Thus on the submerged panels there was an increase in the mean length from 17·2 to 25·8 mm. from October 1950 to October 1951, that is during the second season, and even a slight increase to 26·3 mm. during the third season, whereas in the intertidal exposures the second season's increase in length was of the order of 4 mm. (the maximum mean length reached at the end of the second season was 21·7 mm. on Panel VII and 19·1 mm. on Panel III), the relative increase being greatest on the higher panels where there had been only slow growth in the first season.

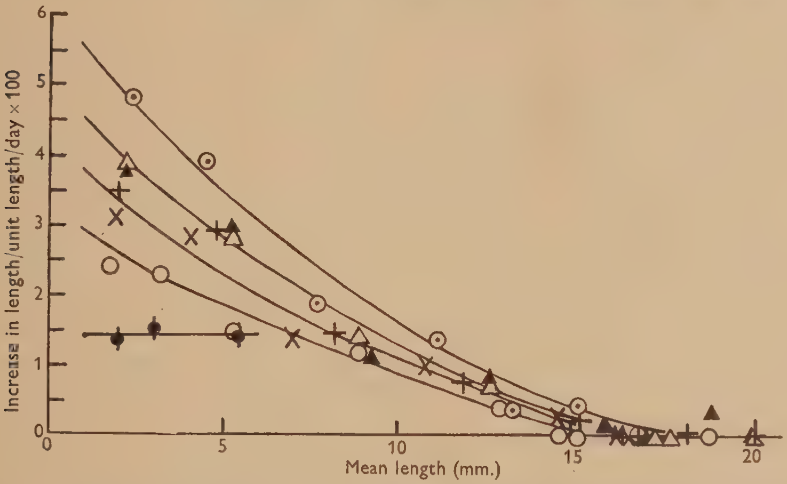

Fig. 5. The mean specific growth-rate (i.e. increase in length per unit length per day) × 100 plotted against mean length, for *B. balanoides* on intertidal Panels II–VII, together with that for the completely submerged specimens for the previous year. Symbols as in Fig. 3; submerged *B. balanoides* shown as dot-and-circle.

The Length, Breadth and Height Relations during Growth

Runnström (1925) has stated that *B. balanoides* at Herdla were relatively higher in the second than in the first year, but he gives no measurements. Moore (1934) observed no change in the apical angle of individuals on the shore, but recorded a change in others growing under estuarine conditions.

The periodic measurement of length, breadth and height of individuals in the present work allows the possibility of such changes in shape to be investigated.

For all raft and intertidal exposures the length/breadth and length/height ratios were calculated for each individual on each separate date of inspection during the growing period; the mean values of the ratios and their standard errors were then found for each set on all the inspections. In Figs. 6 and 7 these mean ratios are plotted against the appropriate mean length: the pooled standard error of the means is also shown. The curves were drawn by inspection.

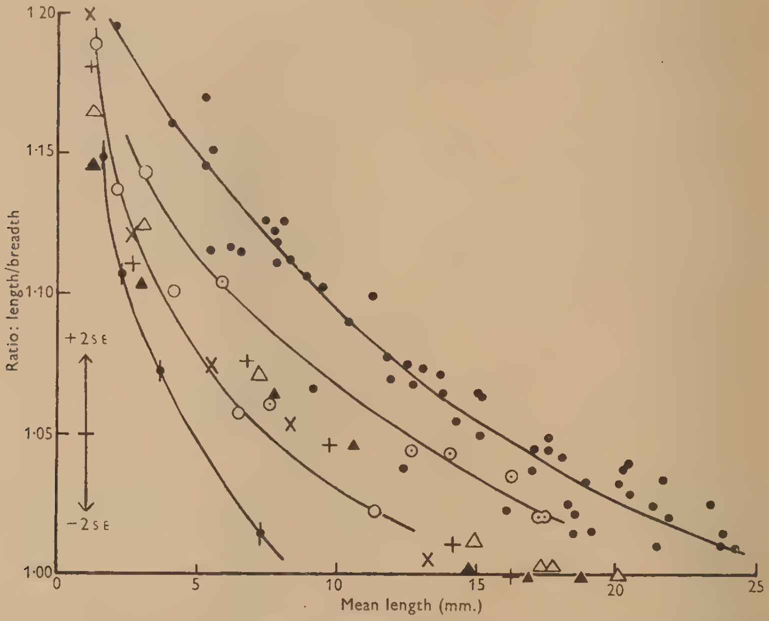

Fig. 6. The mean ratio of length to breadth plotted against mean length for *B. crenatus* totally submerged and *B. balanoides* on intertidal and totally submerged panels. Limits of 2 × standard error shown by arrows. *B. crenatus*, all series shown as black circles, since there was no significant difference in ratio (see text); *B. balanoides*, as in Fig. 5.

No account is taken of age in these Figures. With *B. crenatus*, with its several Series, the barnacles represented for a given mean length are often of quite different total age, as a result of taking into account all the settlements which were spread over several months.

B. crenatus will be considered first. Fig. 6 indicates that the length/breadth ratios decrease with increasing size and, within the limits of random sampling errors, can be considered to lie on a single curve, i.e. there is no indication that the ratio changes from Series to Series: in other words, the ratio is not a function of the time since settlement but only of the size attained by the animal. The rate of change of the ratio with length is greater at the lower mean lengths, i.e. during the period of most vigorous growth (see p. 112). The total change in the mean ratio is not great, from 1·200 at a mean length of 2 mm. to 1·010 at a mean length of 24 mm. In terms of change of shape, this decrease in the ratio with increasing size means that throughout growth the outline of the base changes, most rapidly at first, from more or less elliptical to almost circular. (If the actual cyprid itself were considered then the initial change would be greater.)

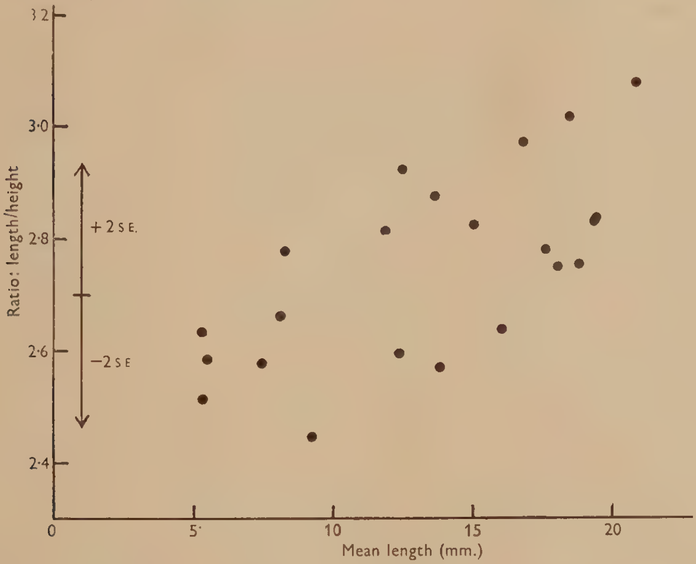

Fig. 7. The mean ratio of length to height plotted against mean length for totally submerged *B. crenatus*. Limits of 2 × standard error shown by arrows.

The method of measuring the height is far less accurate and satisfactory than that of the length and breadth, for the upper part of the compartments, particularly of the carina which was usually measured, is liable to damage. These inaccuracies are reflected in the large standard errors, the pooled value of which was 0·119, compared with a value of 0·014 for the length/breadth ratio. However, quite clearly the ratio increases with increasing mean length, the barnacles becoming relatively squatter as they become older. As far as can be ascertained, the relation is linear throughout the growth of the barnacle.

The form of the curve for length/breadth ratio of submerged *B. balanoides* plotted against mean length is similar to that for *B. crenatus*, that is, there is a decrease in the ratio as the animal increases in size, and the rate of change of the ratio with length decreases with increasing size of the barnacle. Again, therefore, there is a change from an elliptical to a circular outline of the base. The values of the ratio at a given length all lie below those for *B. crenatus* at the same length and a separate curve has therefore been drawn for *B. balanoides*. The position of this curve, below that for *B. crenatus*, implies that at a given length *B. balanoides* is more circular than *B. crenatus*.

The length/height ratios for the submerged *B. balanoides* were too few to graph: the results again showed a wide spread. However, the value of the ratio at a length of 3·1 mm. was 2·42, at 6·0 mm. 3·27, and thereafter there was no significant change. The major change therefore took place very early during growth, unlike *B. crenatus* in which the ratio showed a progressive change.

The results for the intertidal *B. balanoides* are also shown in Fig. 6, which indicates not only that the length/breadth ratios at a given length tend to vary according to the tidal level, but also that the rate of change of the ratios also changes with the level, although clearly the values on Panels V–VII can be considered to lie on a single curve which approaches that for the submerged *B. balanoides* (in the previous season). The difference in Panel II, the highest of the set, is, however, quite striking: at a mean length of 7·25 mm. reached in 98 days (in contrast to a time of 45 days required to reach this length on Panel VII) a ratio of 1·015 is found, whereas this ratio is not reached on the lower panels until a mean length of some 14 mm. has been reached and 19 mm. on the permanently submerged exposures.

According to Darwin (1854) the growth of the shell of a barnacle such as *B. crenatus* takes place in the following way. Accretions to the basis occur only around the circumference, hence increasing the diameter; the compartments grow at their basal margins where they are in contact with the base and hence the shell increases in height; the compartments also grow along their lateral margins, i.e. along the edges of the radii and alae, so that the upper part of the shell also increases in diameter. The foregoing results on the changes in shape imply that there are differential changes in the rate at which calcareous material is laid down during the life of the barnacle. For the length/breadth ratio to decrease during growth there must be a relative increase in growth-rate at the basal margins of the lateral compartments, as well as at the lateral parts of the calcareous basis in *B. crenatus* in order to maintain the continuity of the shell. The relative changes in height, which it should be remembered refer to the carinal compartment, also indicate changes in relative growth-rates in this part of the shell with respect to the overall length.

SUMMARY OF LITERATURE ON GROWTH-RATE OF *BALANUS BALANOIDES* AND *B. CRENATUS*

Runnström (1925), working at Herdla, has given data for the rostro-carinal diameter of intertidal *B. balanoides* over a period of 2 years, making no distinction with regard to tidal levels: the mean spring tidal range, however, is only 4 ft. The settlement took place in late April (29th) and growth followed during the first spring and early summer, but had almost ceased by July, by which time the mean length was only 3·0–4·0 mm. He states that growth began again in October of the first year and by the following January the mean length was 7·0 mm. Further growth apparently took place in the following spring and decreased very rapidly in the summer, at the end of which a mean size of 10·4 mm. had been attained. He states that the first year is a vegetative year and that maturation during the second season is followed by fertilization in October, and the liberation of nauplii in the third spring. Further, he considered that *B. balanoides* lives for two years, occasionally three.

It is clear from Runnström's figures that individual barnacles were not followed, since the numbers measured at each inspection vary irregularly, e.g. on 15 February 1925 twenty-six were measured, while on 7 April 1925 102 were measured; and in view of this fact and the lack of data between July and October in the first season, it is doubtful if the information is sufficient to permit a critical assessment of the growth. Any marked growth during the period October to December of the first season would also seem to be somewhat doubtful. The spat was not followed into the second year but the growth-rate over 2 years was deduced by the measurement of older individuals inferred to be of the second year-class from the different appearance of the shells due to algal infection (cf. Parke & Moore, 1935). Indeed his graph, when the absence of data between 24 June and 15 October is taken into account, can be interpreted to follow quite closely the form of Figs. 2 and 4, with a gradual slowing of the growth in the late summer, although his actual growth-rates at any one time are very much smaller. The only real discrepancy is the period of rapid growth which he suggests takes place in the first mid-winter and which, in view of the great similarity of environmental conditions (see below) and of the method of deriving these figures, i.e. from two entirely different sets of barnacles, may be questioned.

The *B. balanoides* measured by Moore (1934) were natural populations growing at the top, centre and near the bottom of their intertidal range. The growth was not measured from settlement and the actual ages of the barnacles were deduced. Indeed his growth curves extending over 3 years are hypothetical curves based on measurements over 1 year only. However, for the first year he indicates that rostro-carinal lengths of 5·2, 5·3 and 4·7 mm. were reached on his upper, middle and lower patches, respectively. In addition, samples of his O and Q groups (of known age but from a level not specified) were measured and the growth curve for the O group is similar to those of the present investigation. Thus he found rapid growth from April to July, but virtually none during the late summer and winter, and that growth was renewed by May of the second season. There are no results from the early part of the second spring, so it is not known when the second season's growth began. Moore's other group (Q) showed very little growth in the first season, and he suggests that they settled so late that their growth was retarded during the hot summer period.

Hatton & Fischer-Piette (1932) have also studied the growth of intertidal *B. balanoides*, on blocks placed at a number of tidal levels and subject to varying conditions of wave-action, in the St Malo region. The rostro-carinal lengths were measured. Settlement in this region takes place from March to May and the graphs of two individual barnacles measured during two successive seasons (neglecting certain points which show a decrease in size and which are explained as due to erosion) show growth curves similar to those in Figs. 2 and 4. Rapid increase in length took place during the spring and slowed down between July and October. The pattern was repeated in the second season, but growth began between February and March. A length of about 3·0 mm. was reached during the first season and 5·5 mm. by the end of the second season. Further, Hatton & Fischer-Piette point out that the greatest growth is at the lower levels where the animals are immersed for longer periods. Hatton (1938) confirms and extends these observations.

Corlett (1948) reporting on work in the Mersey estuary found that *B. balanoides* settling in April attained a mean rostro-carinal length of 5·9 mm. by December of the first season, and 6·55 mm. by the following April and states that growth was most rapid between May and September. He also records the occurrence of extremely large individuals (20·35 mm. mean length) of unknown age on concrete piles.

Fuller (1946) reports a mean length of 4·0, 6·0 and 8·0 mm. in 4, 8 and 12 weeks respectively, for *B. balanoides* settling between May and July on panels exposed to continuous submersion at Frenchman's Bay, Maine; but he also records that the growth of barnacles settling a year later was much less, approximately 1·0, 1·5 and 2·0 mm. in the same periods. He suggests that the smaller growth on the later occasion was due to the barnacles being situated on the lower surface of deep panels.

There are fewer data available for *B. crenatus*. Pyefinch (1948), working at Millport, has measured samples growing on panels submerged from a raft, and his figures expressing the modal lengths show rapid increase in length during the spring, with a falling off during the late summer and, by interpolation, a rapid growth in autumn of a late settled spat. He finds that the maximum size can be attained in the first season's growth as has been confirmed in the present observations. Topsent (1911) states that at Luc-sur-Mer, apparently in an intertidal, but low zone, *B. crenatus* can attain a size of 3·0–3·5 mm. in 50–60 days, and 5·0–6·0 mm. after 105 days.

Corlett (1948) found that 100 specimens of *B. crenatus*, settled some time between late April and early July 1946, had a mean length of 5·6 mm. (maximum 11·5 mm.) by October 1946; another group had a mean length of 6·6 mm. (maximum 11·0 mm.) in April 1947.

Barnes & Bagenal (1951), studying *B. crenatus* settled on *Nephrops nor-*

vegicus (L.), have deduced that spring-settling *Balanus crenatus* grow to a mean length of 4·4 mm. by October and 8·5 mm. by the following October, while those settling in the autumn showed some growth during the winter and reached a mean length of 1·8 mm. by January and 4·9 mm. by the following July.

Discussion

When due account is taken of the methods of size estimation used by many workers on populations whose age was not accurately known, the results here presented seem to give a picture of the seasonal growth which is valid for all the regions from which results are available, from Herdla to St Malo and Luc-sur-Mer, and in the Gulf of Maine. This result is not surprising since the relative seasonal changes of water temperature and other environmental factors (with the possible exception of summer air temperatures) are not widely different in any of these regions.

Settlement in the spring is followed in both species by a rapid increase in length during late spring and early summer, after which the rate of increase in length falls off in the late summer and autumn and begins again during the following spring. In *B. crenatus*, under conditions of permanent submersion, the early spat appears to be able to reach a virtual maximum size in the first season, that is by October of the first year, and the small growth taking place in the second season begins somewhat later than in the first. In *B. balanoides*, however, on submerged panels, the maximum length is not reached in the first season, and since the specific growth-rate is higher in the smaller animals growth continues further into the winter, and starts earlier and is more vigorous than in *B. crenatus* during the second season.

There is no doubt from a comparison of the present results with those summarized above that, when growing free from competitive and other restricting environmental factors, *B. balanoides* grows more rapidly under conditions of complete immersion, at any rate when the constant depth is no greater than 3 ft. The evidence from the intertidal exposures suggests that at any level the maximum size reached is approximately the same, since by the end of the second season there is little difference at the five levels. This was a conclusion reached by Hatton & Fischer-Piette (1932) in studying *B. balanoides* settled on blocks exposed intertidally. It seems, therefore, that the observation of Moore (1935*b*) and of Hatton (1938) that the optimum level for growth is at low water for newly settled barnacles, but moves progressively up to high water as they grow older, is due in part to the specific growth-rate varying with size. The older barnacles at the top of their distribution will be growing more in their second season simply because they have grown slowly during the first, although other environmental factors, present on the shore and absent on intertidal panels kept relatively free from other growth, may play a part.

Quite evidently it is not the unsuitability of complete submersion for growth and development that usually limits *B. balanoides* to the intertidal zone.

At all levels on the intertidal exposures, observations on barnacles other than those measured showed that maturation followed by fertilization and development of nauplii had taken place during the first season. Fertilization of *B. balanoides* in this area takes place in the autumn when the lowest mean size attained (by those on Panel III) was between 11·0 and 14·0 mm. Other work has shown that, even though growth is much less, on the adjacent shore barnacles produce nauplii during their first year at all levels. It seems that the vegetative year observed by Runnström (1925) is due to the very low growth-rate, indicated by the small size reached at the end of the first summer's growth (some 3 mm.). The observations of Moore (1935*a*) that the barnacles at the lower tidal levels matured in their first year, but at higher levels not until a year later, could possibly also be explained in this way. Again, the *B. balanoides* measured by Corlett (1948) had only reached a mean length of 5·9 mm. in December, and he records that rather less than 10% contained developing nauplii. Neither Moore nor Corlett, however, indicate whether the sterile barnacles in question were in fact sufficiently close together to permit cross-fertilization. In our observations the high-level *B. balanoides* on both the pier panels and on the shore were close enough to have been fertilized by adjacent barnacles, but frequently high-level barnacles are so scattered as to be incapable of cross-fertilization.

Further, under the conditions of these experiments, far greater sizes were attained than are usually recorded under what may be termed more natural conditions. Occasionally isolated and presumably very old individuals are found at the upper levels on the adjacent pier piles (see also Corlett), the size of which approaches those recorded here. Presumably, therefore, under natural competitive conditions death frequently intervenes before the possible maximum size is reached. It should, of course, be emphasized that, from the point of view of continued survival of the barnacle population as a whole, the isolated individuals here considered are atypical since cross-fertilization would never be achieved.

The exceptional growth of *B. crenatus* on submerged panels might be expected since it is normally a sublittoral but not a deep-water species. The sizes recorded by Barnes & Bagenal (1951), and indeed general experience of *B. crenatus* in the field, indicate that these maximum possible sizes are not normally reached. The environmental factors, perhaps in particular food supply, seem to be more favourable under raft conditions.

The fact that the specific growth-rates for all the Group I *B. crenatus* lie on a single curve, although there were 2 months between the times of settlement of Series I and IV, together with the lack of marked discontinuities in the curve, suggests that environmental conditions which varied throughout this period were not limiting the growth-rate. The possibility, however, cannot be ruled out that there were two different environmental factors acting in opposition. Thus, the sea temperature when Series I had reached a mean length of 12·0 mm. was 11·0° C., but was 15° C. when Series IV were growing at this mean length (see Fig. 1), and, although the specific growth-rates were identical, possibly an increased food supply at the lower temperature in June was equivalent to a reduced food supply for Series IV at the end of July with its higher temperature. There is some evidence that food supply becomes a limiting factor later in the season, since Series V in August, with the temperatures still comparatively high, had lower specific growth-rates than expected (from Group I data) at their mean length. Indeed the lower specific growth-rates for Group II as a whole, between August and October, even though the temperatures during this period were always higher than those in the spring, suggest that food supply may then have been a controlling factor. Lack of food also seems to be a major factor affecting growth during the winter period since the mean temperatures during January are almost as high as those during the spring, an opinion further substantiated by the increase in the specific growth-rate during the following March, when food becomes available with the advent of the spring diatom increase.

SUMMARY

The length, breadth, and in part the height, of individual *Balanus balanoides* and *B. crenatus* have been measured over several seasons, and the mean specific growth-rates calculated. The *B. balanoides* had settled at various known intertidal levels, and both species under conditions of permanent submersion.

Under all conditions, except on the highest intertidal panels, length increases rapidly subsequent to settlement and during the early summer. *B. crenatus* can reach its virtual maximum size (20–25 mm. rostro-carinal length) in a single growing season, under the conditions studied. Late-settled *B. crenatus* continue to grow further into the autumn, and begin to grow again early in the spring of the second season at the end of which the virtual maximum size is reached. The seasonal growth of both intertidal and submerged *B. balanoides* is similar, but the growth-rate is a function of submersion, which is, of course, dependent upon the tidal level. However, maximum growth is not reached in the first season and there is consequently relatively more growth in the second season than in *B. crenatus*.

The mean specific growth-rates in both species decrease with increasing size and, with permanently submerged *B. crenatus*, there is no suggestion that during the first spring and early summer any change in the environmental factors was effective. On the intertidal panels, the mean specific growth-rates at a given size vary with the tidal level, and this effect is most marked in the smaller barnacles.

The ratio of length to breadth decreases, at first rapidly, then more slowly, with increase in length in both species, so that the outline of the base approaches that of a circle. The relation varies according to the tidal level. The height measurements were less satisfactory, but it is evident that the ratio increases with increasing size, the barnacles becoming relatively squatter.

The net settlement of *B. balanoides* on the intertidal panels was maximal at about mid-tide level, and it is suggested that this is the result of two opposing factors, namely, gain due to settlement when the tide *crosses* the panel and loss due to desiccation during exposure to air. The hypothesis is based upon the suggestion that the cyprids of an intertidal species such as *B. balanoides* settle most readily from relatively thin layers of water draining from solid surfaces.

A high incidence of infection by *Hemioniscus balani* was noted in the intertidal *B. balanoides* in the second season.

A comparison with the results of other workers shows that the growth-rates recorded and sizes reached on these experimental exposures are far greater than have hitherto been observed under natural conditions, although the seasonal growth cycle is similar over a wide area. In *B. balanoides* it is clearly not the unsuitability of complete submersion for growth and development that usually limits this species to the intertidal zone.

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COMPARISON OF THE DIFFERENT METHODS OF ESTIMATING NANOPLANKTON

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An assessment of the abundance of nanoplankton in the sea cannot be made until a reliable method for counting has been found. The object of the present investigation was to compare the efficiency of a number of methods. The organisms concerned range in size from less than 1 to about 10 μ, most being less than 5 μ, and belong to the classes Chrysophyceae, Cryptophyceae, Dinophyceae and Chlorophyceae.

The larger planktonic diatoms and armoured dinoflagellates, which occur in the plankton in numbers insufficient to give accurate results with the methods under comparison, have been omitted. Utermöhl's (1931) sedimentation method gives the best results for these larger forms (Nielsen, 1933); but it is not suitable for naked nanoplankton, since no adequate fixative has been discovered which will leave these organisms in a state recognizable for identification. A second method, suggested by Goldberg, Baker & Fox (1952), of filtering samples through a molecular filter, is convenient for treating all size-ranges of organisms at sea. Counts may then be made in the laboratory without any prior treatment of the sample, but the majority of the naked forms up to 5 μ are unidentifiable.

Some means of concentrating the sample is usually necessary in most counting methods since the depth of the water film which would be required to give any figure at all from a raw sample is too thick to allow an accurate count. This statement does not apply to Dr R. H. Millar's technique of counting under dark-ground illumination.

The methods compared here are the use of either a centrifuge or filters for concentrating the sample, followed by counting; a counting method using dark-ground illumination without previous concentration of the sample; and the utilization of dilution techniques.

My most sincere thanks are due to Dr M. Parke, of Plymouth, for her many helpful suggestions during the course of this investigation; to Dr R. H. Millar, of Millport, to whom I am indebted for an account of his method for counting flagellates under dark-ground illumination; and to Dr E. D. Goldberg, of the Scripps Institution of Oceanography, California, for a description of the use of the molecular filter, and for a supply of these filter membranes.

My thanks are also due to the Director, Mr F. S. Russell, and staff of the Laboratory of the Marine Biological Association at Plymouth, where this work was carried out when the author was in receipt of a Development Commission Fisheries Training Grant.

METHODS

Much has been said in the past about the use of a centrifuge for concentrating nanoplankton samples, and many different methods have been used. Gran (1929) states that 'test experiments with the centrifuge method show that the source of error involved by vortex formation in the tubes, as mentioned by Wulff (1926) and Utermöhl (1927), can be avoided altogether if care is taken first to ensure that the motion is not too suddenly checked, and tubes with a narrow bottom part used, and the clear liquid drawn up cautiously with a pipette'. The buoyancy of certain species (Kofoid, 1897; Gross & Zeuthen, 1948), due to a specific gravity less than that of water, causes selective action with the centrifuge, but the majority of such species are diatoms or water-bloom-forming organisms such as blue-green algae, which can be estimated with the sedimentation method after fixation.

The method of centrifugation adopted here is based upon that of Bruce, Knight & Parke (1940). 10 ml. samples are centrifuged for 15–30 min. at 1500 r.p.m., a relative centrifugal force of 358 × gravity. The centrifuge is then stopped very slowly, to avoid currents developing in the tubes, and the top 9 ml. are withdrawn by means of an upturned pipette attached to a pump. The use of the pump and a pipette with an upturned end enables the water to be drawn off evenly, and any suction currents which may develop will be only at the surface of the water. The remaining 1 ml. of water is then thoroughly mixed, and counts made in a haemacytometer, objectives up to $\frac{1}{6}$ in. being used if necessary. Average values, based on counts from four tubes, were recorded for each sample.

Several experiments have been carried out to determine the speed and duration of centrifugation which gives the maximum results (Table I). In these experiments no attempt was made to count all the organisms in the concentrate, as Lohmann (1908) did, but an average figure was obtained for each sample.

Nielsen & v. Brand (1933–34) describe the use of a precipitate to aid in the sedimentation of preserved cells while centrifugation is in progress, and it was thought that this principle might be applicable to the centrifugation of living cells. Accordingly, before centrifugation, 0·05 ml. of a 1% solution of potassium aluminium sulphate crystals was added to the 10 ml. sample of sea water (Atkins & Parke, 1951). The flocculent precipitate thus formed does not kill the organisms, nor does it hinder subsequent counting. Further treatment of the precipitate is therefore unnecessary. The results from this method using the floc, compared with results from untreated samples (labelled 'normal') are found in Table I, Sample 1, p. 134, and Table IV, samples 2 and 3, p. 137.

As a check on the sedimentation of living organisms during centrifugation 10 ml. samples were centrifuged and split up immediately into three sections (i.e. the top 5 ml., middle 4 ml. and bottom 1 ml., the latter being the section usually used for estimating the numbers). These separate sections were then placed in dilute Erdschreiber[1] culture solution and left to grow. Never did more than two different organisms appear in the flask containing the top 5 ml. of the centrifuged sample, or more than three in the flask containing the middle 4 ml., and the time taken for these cultures to develop was comparable with that for cultures started from a single-cell inoculum at the same period of the year. The flask containing the bottom 1 ml. of the sample, however, produced a thick mixed culture in a short time. This evidence, together with that obtained from centrifuging cultures (Table II), proves that the bulk of living nanoplankton organisms will settle during centrifugation.

The second method of concentrating sea-water samples was by means of the 'Stefi' filter, using a collodion membrane having an average pore diameter of 0·5 μ (Cole & Knight-Jones, 1949). With this apparatus samples were concentrated to one-fiftieth of their original volume, and samples of the concentrate were counted in a haemacytometer.

To facilitate the counting of a large number of motile organisms under direct illumination, fixation with osmic vapour is necessary. This treatment leaves the organisms in a recognizable state, but cannot be used for large volumes of water. The percentage of motile and non-motile forms in each size-group can be determined later if required.

The third method tested for concentrating the nanoplankton was by means of the molecular filter suggested by Goldberg *et al.* (1952). This filter-membrane is composed of incompletely cross-linked high polymer molecules of partially substituted cellulose acetate and cellulose nitrate. The filters used in this work had an average pore-diameter of 0·5 μ. Following the method of Goldberg *et al.* all solutions used in the preparation of the sample were molecular-filtered before use to remove any particles. Organisms in the sample were then fixed by means of a saturated solution of iodine in potassium iodide, at a concentration of 40 ml. of fixative to a litre of sea water. Throughout, samples of 50 ml. were used. After fixation the sample was filtered, and the sea water was gradually replaced by distilled water by washing with a series of sea water/distilled water mixtures. 20 ml. of Fast Green were added to the filter, which was then disconnected from the pump, and left for 30 min. The Fast Green was then drawn through, and the membrane was dehydrated with ethyl alcohol and cleared with cedar oil.

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[1] Dilute Erdschreiber culture solution (Plymouth formula): sea water 1 l., $NaNO_3$ 0·15 g., $Na_2HPO_4 . 12H_2O$ 0·015 g., soil extract 25 ml.

The membrane was then removed from the filter, cut in half, and each half mounted in balsam on a microscope slide. If the number of diatoms and larger dinoflagellates in the sample is required, the entire area of the filter is used. These organisms can usually be easily identified on the membrane. To estimate the numbers of nanoplankton four fields only were counted, each being taken at random from the filtering area of the membrane. The mean of these counts was multiplied by 83 × 95/50,000 to bring the figure to numbers per cubic millimetre of the original sample. This correction factor must be calculated for each filtering apparatus and microscope used when counting.

The next method (unpublished), designed and used by Dr R. H. Millar of Millport, for the counting of flagellates, makes use of the highly refractive nature of the nanoplankton organisms. The details of this method have been communicated to me by Dr Millar, for use in these comparative studies. In his method a drop of water is placed in the cell of a counting slide (Thoma haemacytometer), and, with a phase-contrast microscope adjusted to give dark-ground illumination with a low-power objective, all the moving flagellates are rapidly counted in the whole area of the cell, which is scanned by means of a mechanical stage. Any microscope fitted with dark-ground equipment can be used for this method, but the advantage of a phase-contrast microscope is the ease with which it can be altered to give either direct illumination, or actual phase contrast, so that particular organisms can be observed more clearly. The count must be made as soon as possible after the drop of water is placed on the slide, as many organisms settle very rapidly.

The next two methods tested for estimating nanoplankton involve the use of cultures. The first was proposed by Allen (1919) and the second by Knight-Jones (1951). Allen's method consists simply of adding 0·5 ml. of the sea-water sample to 1·5 l. culture solution, shaking thoroughly, and distributing this into a large number of flasks, which are then left in a north light for the organisms to grow. It is then assumed that every species which grows in each flask was started from a single cell, thus giving a minimal count. Knight Jones's method utilizes the serial dilution technique used by bacteriologists, with Erdschreiber culture solution as the nutrient medium. Three series of five tubes are used in each estimation, each series containing one-tenth of the volume of the sample that is present in the previous series. The tubes are placed in a north light and left to grow for 1–2 months in the summer, and longer in the winter. On the basis of the number of tubes in each series which show growth the final results are worked out from Swaroop's (1938) tables of the most probable numbers in the original sample. This serial dilution method has been used with sea-water samples, where the numbers obtained have been compared with counts after concentrating with the membrane filter and centrifuge, and also with actively growing cultures, where the number of cells was counted prior to dilution.

RESULTS

Centrifugation

(Tables I, II, IV sample 2, VIII, and X sample 2)

The experiments to test the efficiency of centrifugation at different speeds and for different periods of time were carried out using both samples treated with potassium alum and untreated ('normal') samples. The results of these experiments are shown in Table I and the figures from which they were obtained are in Table VIII (p. 143).

Sample 1 shows that there is no significant difference between untreated samples and those which have been treated with potassium alum. This conclusion is confirmed by results shown in Table IV (p. 137), and from other results not given here, including some from experiments using cultures.

Results of centrifugation for different periods at 1500 r.p.m. (Sample 1) show that a 10 min. period does not give a maximum count, but from 15 min. onwards there is no significant increase. Increasing the centrifuge speed to 2000 r.p.m. does not improve the efficiency of sedimentation (Samples 2 and 3).

After the relative centrifugal force and time above which no further sedimentation occurs had been established, the actual efficiency of centrifugation was tested by the use of cultures. Cultures of a density that permitted an accurate count in a haemacytometer were centrifuged for 20–30 min., and counted. The results from these experiments are shown in Table II. The culture had usually to be diluted to one-tenth of its original strength prior to centrifugation, as the excessive number of organisms after concentration would otherwise have precluded an accurate count.

Throughout Table II the expected standard deviation (σ), based on the Poisson distribution, has been given, the actual standard deviation being unknown. Where thick cultures were used in this series of experiments a lower count than that obtained from the original sample resulted after centrifugation. This was particularly true with flagellate no. 3 (Plymouth collection) and *Hemiselmis rufescens*. In *Hemiselmis* the count from the centrifuged diluted culture is considerably larger than that from the centrifuged undiluted culture, and it seems possible that the density of the culture limits the efficiency of sedimentation during centrifugation.

These results strongly suggest that the centrifuge sediments the great majority of the nanoplankton, particularly in samples in which the organisms are not very numerous, as in raw sea water itself.

Centrifugation and membrane filter

(Tables III and IX)

Table III shows the comparison of results obtained by counting after samples had been concentrated with the membrane filter and the centrifuge.

TABLE I. COMPARISON OF SPEEDS AND TIMES OF CENTRIFUGATION

(Figures throughout refer to numbers per cubic millimetre, and results marked * are significantly different.)

Sample 1: 1500 r.p.m. (relative centrifugal force = 358)

Duration (min.)	'Normal' or 'Floc'	Mean	Standard deviation (*s*)	Difference between means	Standard error of difference
10	N	7·25	1·83		
	F	7·62	2·98	0·37	±1·24
15	N	10·25	3·18		
	F	9·9	3·04	0·35	±1·55
30	N	11·25	2·71		
	F	10·0	2·4	1·25	±1·28
60	N	10·0	2·83		

Treatments compared	'Normal' or 'Floc'	Difference between means	Standard error of difference
10 and 15 min.	N	3·0	±1·34*
	F	2·28	±1·51
15 and 30 min.	N	1·0	±1·475
	F	0·1	±1·42
30 and 60 min.	N	1·25	±1·38
10 and 30 min.	N	4·0	±1·15*
15 and 60 min.	N	0·25	±1·5

As there is no significant difference between 'normal' and 'floc' in any series, these two sets of figures have been treated together for each length of time, and the following results are obtained.

Duration (min.)	Mean	Standard deviation
10	7·44	2·36
15	10·1	3·0
30	10·62	2·56
60	10·0	2·83

Treatments compared	Difference between means	Standard error of difference
10 and 15 min.	2·66	±0·96*
15 and 30 min.	0·52	±0·99
30 and 60 min.	0·62	±1·19
10 and 30 min.	3·18	±0·87*
15 and 60 min.	0·1	±1·25

Sample 2: collected from Knap Buoy, 3 June 1952

Treatment	Mean	Standard deviation (*s*)
1500 r.p.m. for 15 min.	25·75	2·49
2000 r.p.m. for 15 min.	25·22	3·42
2000 r.p.m. for 30 min.	25·4	4·11

Sample 3: 15 min. at 1500 and at 2000 r.p.m. compared

Speed in r.p.m.	Mean	Standard deviation	Difference between means	Standard error of difference
1500	10·4	2·45		
2000	9·0	2·07	1·4	±1·12

N.B. At 2000 r.p.m. the relative centrifugal force is equal to 636 × gravity.

It will be seen that the theoretical Poisson standard deviation, the root of the mean, is frequently much less than the observed standard deviation for membrane-filtered samples, a fact which suggests that the organisms tend to aggregate when subjected to this treatment. χ^2 has then been calculated, and the probability found from Tables. This figure is often very low. This evidence of aggregation is rarely found in centrifuged samples. In all the results from centrifuged samples there is only one (Table III, sample 4) in which the probability is less than 0·05, a result well within chance expectations. The counts from which these figures are derived will be found in Table IX (p. 144).

TABLE II. RESULTS FROM THE CENTRIFUGATION OF CULTURES

(Centrifugation at 1500 r.p.m. for 20–30 min. The results are expressed per cubic millimetre of the original culture.)

	Original count		Culture centrifuged		Culture diluted and centrifuged	
Organism	Mean	Theoretical standard error (σ)	Mean	Theoretical standard error (σ)	Mean	Theoretical standard error (σ)
Chlorophyceae						
Chlamydomonas sp. 1	4440	221	..	..	4300	208
Pyramimonas grossii Parke	220	47	..	..	200	44·7
Chrysophyceae						
Flagellate no. 3	3230	180	..	..	2760	167
Chromulina pleiades Parke	7770	279	..	..	7310	270
Cryptophyceae						
Flagellate no. 6	5300	230	..	..	4900	222
Hemiselmis rufescens Parke	3420	186	2800	53	3210	179
Dinophyceae						
Peridinium trochoideum (Stein) Lemm.	143	37·8	151	12·6	146	38·2
Gymnodinium sp. 2	180	42·5	..	..	190	43·2

The figures in Table III show little, if any, difference between the actual results from the two methods of concentration, except for the sample taken on 3 June 1952, which was full of detritus. The time involved in using the two methods is, however, very different. The centrifuge is simple to operate, and the counts can be made fairly rapidly, as there are only twenty or thirty organisms per field, whereas with the membrane filter great care must be taken to ensure that the membrane does not dry out completely, for in so doing a number of the naked flagellates would be destroyed. The number of organisms per field after this latter treatment is also large, and the time taken to count them is therefore much greater. The additional time spent on the method appears not to be justified by any greater, or more reliable, estimate of the number of organisms.

Table III. Comparison of the Results obtained by Counting after Concentrating with the Membrane Filter and the Centrifuge

(All samples are from Knap Buoy.)

	Counts per haemacytometer field					Numbers per cubic millimetre of the original sample	
	Mean	Standard deviation (*s*)	Deviation (σ)	χ^2	Probability	Mean	*s*
			Sample 1, 27. v. 52				
Membrane filter							
Total	101·7	20·95	10·1	21·5	< 0·001	20·3	4·19
Under 2 μ	64·6	15·95	8·04	19·6	0·01–0·001	12·9	3·19
2–under 5 μ	28·7	6·15	5·35	..	..	5·7	1·23
Over 5 μ	8·3	2·34	2·88	..	..	1·7	0·47
Centrifuge							
Total	20·13	4·375	4·58	..	..	20·13	4·375
Under 2 μ	9·19	2·4	3·03	..	..	9·19	2·4
2–under 5 μ	7·75	2·98	2·78	..	..	7·75	2·98
Over 5 μ	3·19	1·56	1·79	..	..	3·19	1·56
Serial dilution	..	..	..	..	..	3·9	2·1
			Sample 2, 29. v. 52				
Membrane filter							
Total	89·7	16·8	9·7	15·7	0·01–0·001	17·94	3·36
Under 2 μ	53·9	9·7	7·35	..	..	10·78	1·94
2–under 5 μ	25·0	4·52	5·0	..	..	5·0	0·9
Over 5 μ	10·8	5·17	3·29	12·3	0·05–0·02	2·16	1·03
Centrifuge							
Total	22·0	4·76	4·68	..	..	22·0	4·76
Under 2 μ	11·12	2·25	3·34	..	..	11·12	2·25
2–under 5 μ	6·63	2·78	2·58	..	..	6·63	2·78
Over 5 μ	4·25	1·895	2·06	..	..	4·25	1·895
Serial dilution	..	..	..	..	..	2·0	1·0
			Sample 3, 3. vi. 52				
Membrane filter							
Total	202	16·9	14·2	..	..	40·4	3·4
Under 2 μ	131	17·4	11·45	11·5	0·05–0·02	26·2	3·5
2–under 5 μ	44·3	18·5	6·66	38·6	< 0·001	8·9	3·7
Over 5 μ	26·7	5·46	5·17	..	..	5·3	1·09
Centrifuge							
Total	33·1	6·77	5·75	..	..	33·1	6·77
Under 2 μ	21·0	4·62	4·58	..	..	21·0	4·62
2–under 5 μ	6·7	2·33	2·59	..	..	6·7	2·33
Over 5 μ	5·4	1·96	2·32	..	..	5·4	1·96
N.B. This sample was full of detritus, and very difficult to count, and this fact may account for the divergent results.							
			Sample 4, 6. vi. 52				
Membrane filter							
Total	173·0	24·75	13·2	17·7	0·01–0·001	34·6	4·95
Under 2 μ	124·0	20·6	11·3	17·01	0·01–0·001	24·8	4·12
2–under 5 μ	26·0	6·32	5·1	..	..	5·2	1·26
Over 5 μ	23·0	5·43	4·8	..	..	4·6	1·1
Centrifuge							
Total	36·0	8·57	6·0	14·22	0·05–0·02	36·0	8·57
Under 2 μ	25·9	6·86	5·1	..	..	25·9	6·86
2–under 5 μ	4·5	2·0	2·12	..	..	4·5	2·0
Over 5 μ	5·6	2·51	2·37	..	..	5·6	2·51
			Sample 5, 9. vi. 52				
Membrane filter							
Total	135·9	38·7	11·7	55·0	< 0·001	27·18	7·7
Under 2 μ	100·5	32·7	10·03	53·0	< 0·001	20·1	6·5
2–under 5 μ	23·7	5·27	4·87	..	..	4·74	1·05
Over 5 μ	11·7	3·88	3·42	..	..	2·34	0·78
Centrifuge							
Total	27·1	5·35	5·21	..	..	27·1	5·35
Under 2 μ	19·0	3·02	4·36	..	..	19·0	3·02
2–under 5 μ	4·7	2·45	2·17	..	..	4·7	2·45
Over 5 μ	3·4	1·65	1·84	..	..	3·4	1·65

Centrifugation, membrane filter, dilution techniques

(Tables III samples 1 and 2, IV, V, IX samples 1 and 2, X, and XI)

In Table III, samples 1 and 2 show a third estimate of numbers, obtained by the serial dilution method. Further figures obtained from sea-water samples by this method are shown in Table IV, in which the figures are derived from counts shown in Table X (p. 145).

TABLE IV. TABLE OF RESULTS FROM SAMPLES ESTIMATED BY (*a*) CENTRIFUGATION, FOLLOWED BY COUNTING IN A HAEMACYTOMETER, AND (*b*) BY DILUTION

(Numbers are given per cubic millimetre of the original sample.)

Method	Mean	Standard deviation	Difference between means	Standard error of difference
	Sample 1, 9. iv. 51			
Centrifuge	32·0	8·26	..	..
Serial dilution	0·79	0·45	..	..
Allen's dilution	0·204	..	..	..
	Sample 2, 2. iv. 52			
Centrifuge 'Normal'	8·12	1·46	0·88	±0·66
Centrifuge 'Floc'	9·0	0·815		
Serial dilution	2·1	1·0	...	..
	Sample 3, 9. iv. 52			
Centrifuge 'Normal'	9·5	2·93	2·0	±1·48
Centrifuge 'Floc'	11·5	2·83		
Serial dilution	1·3	0·7	..	..

Tables III and IV show that the estimates from the dilution methods are extremely low. It appears that this method of estimation cannot be generally applied until a more suitable culture solution and optimum cultural conditions for all the organisms are available—that is, if it is desired to estimate the total number of organisms in the phytoplankton. Dr H. A. Cole, of Conway (private communication), states that 'We have found that figures for the density of flagellates in our tanks, obtained by direct counts of concentrated samples using the membrane filter technique, are almost invariably between about four and ten times those obtained by serial dilutions.'

Further corroboration of this fact is found in Table V, which shows the results of serial dilutions set up using cultures. The daily counts from these cultures over the period when the dilutions were set up show them all to be in a state of active growth (see Table XI, p. 145).

In this series of experiments, as in those using sea-water samples, a suitable preliminary dilution was made before the serial dilutions were set up. This preliminary dilution varied, 1 ml. of the sample to 100 ml. culture solution for a sea-water sample, and 1 : 10,000 for cultures, except *Pyramimonas*, where the preliminary dilution was 1 : 1000.

With the exception of flagellate K, a chrysomonad with three flagella, the numbers obtained by the dilution method were very low throughout these experiments.

TABLE V. THE RESULTS OF THE SERIAL DILUTION TECHNIQUE USED ON CULTURES OF FLAGELLATES, COMPARED WITH THE KNOWN COUNTS FROM THE CULTURES PRIOR TO DILUTION

(Results are expressed per cubic millimetre of the original culture.)

	Count from culture		Dilution method	
Organism	Mean	Expected σ	Most probable no.	σ
Pyramimonas sp.	90	30	39	21
Gymnodinium sp. 2	200	44·8	63	37
Pseudopedinella sp. 1	180	42·5	18	9
Flagellate K	340	58·4	240	121
Flagellate no. 16	255	50·5	33	17

TABLE VI. COMPARISON OF THE RESULTS OBTAINED BY CONCENTRATING SAMPLES BY CENTRIFUGATION AND THE USE OF THE MOLECULAR FILTER

(All samples are from Knap Buoy, and counts are expressed per cubic millimetre of the original sample.)

Sample		Centrifuge		Molecular filter	
		Mean	*s*	Mean	*s*
13. xi. 52.	Total	20·0	3·8	18·06	3·97
	Under 2 μ	12·5	2·64	14·85	3·0
	2–under 5 μ	4·3	1·38	2·09	0·32
	Over 5 μ	3·2	1·74	1·12	0·223
19. xi. 52.	Total	19·8	4·76	16·8	2·1
	Under 2 μ	14·3	3·4	14·2	1·91
	2–under 5 μ	3·5	1·35	1·85	0·395
	Over 5 μ	2·0	0·95	0·75	0·199
21. xi. 52.	Total	21·8	4·06	21·6	1·35
	Under 2 μ	14·4	3·44	18·1	0·9
	2–under 5 μ	4·9	1·6	2·6	0·58
	Over 5 μ	2·5	0·975	0·9	0·25
28. xi. 52.	Total	24·3	4·94	22·8	0·94
	Under 2 μ	16·3	3·44	16·9	0·92
	2–under 5 μ	5·8	1·83	4·55	0·47
	Over 5 μ	2·2	1·475	1·35	0·42
4. xii. 52.	Total	23·3	3·06	24·6	1·65
	Under 2 μ	15·4	3·47	19·98	1·79
	2–under 5 μ	4·8	1·34	3·0	0·56
	Over 5 μ	3·1	1·2	1·62	0·152

Centrifugation, molecular filter
(Tables VI and XII)

Results from the use of the molecular filter are very similar to those obtained after centrifugation. The figures in Table VI showing these results are derived from counts given in Table XII (p. 146).

This method has certain advantages in that diatoms may be estimated in the same sample as nanoplankton, and that the sample may be made into a permanent preparation which can be counted at a later date. Its disadvantages are two, namely that for routine sampling the time involved in the preparation of a sample for counting is about 3 hr., and the actual counting is rather difficult, as the naked forms tend to shrink after killing, and so make identification difficult, if not impossible.

Centrifugation, Dr Millar's method

(Tables VII and XIII)

The last method used in this comparative study is the use of dark-ground illumination for counting an unconcentrated sample. The Thoma cell used has a total volume of 8·66 cubic millimetres, and this is the volume of water in which organisms were counted. The figures for these counts, together with those from samples after centrifuging, will be found in Table XIII (p. 147).

The results from the counts of cultures, given in the first part of Table VII, show that when only motile cells were counted under dark-ground illumination a large number of the individuals, at least of the two species used, were missed, because they had become non-motile. In other samples when both motile and non-motile cells were counted, a figure was obtained which was very near to the count from the culture prior to dilution. The counting of non-motile cells does, however, need considerable care, because a speck of detritus the same size as an organism will produce an image very similar to that of a living cell under dark-ground illumination.

Throughout the estimations of sea-water samples by this method, the results of which are given in the second part of Table VII, both motile and non-motile forms have been counted, and it can be seen that the agreement between the results obtained by the two methods is very close.

DISCUSSION

In considering the relative merits of the above six methods for estimating nanoplankton the ultimate aim of the investigation must be clearly defined. There is very little difference between the efficiencies of the four counting methods with regard to the final estimate of numbers given. If the purpose is to determine only the total number of organisms in a sample of sea water, then counting under dark-ground illumination is the most rapid method. Counts after concentrating with the centrifuge or filters will also give this figure. If, however, the aim is to count the organisms, place them in size groups, and assign them as far as possible to their systematic position, the centrifuge or membrane filter for concentrating living samples should be used. Centrifugation is better because of its speed, and the uniformity of the subsequent counts, as there is no evidence of aggregation of the organisms after this treatment, as there was with the membrane filter.

Table VII. Comparison of Counts using dark-ground Illumination with Counts from Cultures and after Concentration by Centrifugation

(a) Results obtained from cultures

(Numbers are per 0·1 mm.^{3}.)

Organism	Straight count in haemacytometer	Dark-ground counts: Motile only	Dark-ground counts: Motile and non-motile
Hemiselmis rufescens Parke	189	138	179
Chromulina pusilla Butcher	426	230	450

In both cases the cultures were diluted prior to the dark-ground count: the dilution was 1:50 in the case of *Hemiselmis*, and 1:100 in the case of *Chromulina*.

(b) Results obtained from sea-water samples

(Numbers per cubic millimetre of the original sample.)

Sample	Size-group	Centrifuge and count	Dark-ground count
10. xii. 52. West end of breakwater	Total	17·6	16·3
	Under 2 μ	9·9	10·3
	2–under 5 μ	4·5	3·7
	5–under 10 μ	2·3	1·5
	Over 10 μ	0·9	0·8
11. xii. 52.	Total	19·3	19·2
	Under 2 μ	10·1	11·7
	2–under 5 μ	6·1	4·5
	5–under 10 μ	2·2	2·08
	Over 10 μ	0·9	0·92
12. xii. 52. Tank water from the Aquarium	Total	6·4	7·75
	Under 2 μ	4·3	5·15
	2–under 5 μ	1·2	1·62
	5–under 10 μ	0·5	0·64
	Over 10 μ	0·4	0·34
15. xii. 52. Knap Buoy	Total	11·4	10·2
	Under 2 μ	7·4	6·2
	2–under 5 μ	2·5	2·2
	5–under 10 μ	1·1	1·16
	Over 10 μ	0·4	0·64

Sample		Straight count	Diluted to 1/10 prior to dark-ground count
Mixed culture of diatoms and flagellates	Total	11·6	13·4
	Under 5 μ	7·9	8·9
	5–under 10 μ	2·6	3·1
	Over 10 μ	1·1	1·4

In this last sample the numbers are given per 0·1 mm.^{3}.

The dilution methods give very low estimates of the numbers of nanoplankton in sea water, and the time elapsing between setting up the dilutions and the end of the period required for the organisms to give a reasonably thick culture may be several months. This makes the method cumbersome if a large number of samples are to be estimated.

The importance of cultures of nanoplankton should not, however, be underestimated, in view of the numbers of hitherto undescribed species which occur in sea water. It is very necessary to pick out organisms and grow them in species-pure culture in order to study them more closely. The two dilution methods can also be of great use in isolating certain organisms, as they save the tedious labour involved in isolating individual organisms by means of a micropipette.

SUMMARY

Six methods for estimating the numbers of nanoplankton organisms in sea water have been compared. Of these, centrifugation of a living sample, followed by counts of the numbers of organisms in the concentrate, appears to be the most satisfactory method from all points of view. The method is rapid, simple to operate, and gives a result which is strictly comparable with results from counts made under dark-ground illumination of unconcentrated samples, and from counts after concentrating with filters. By this method the organisms counted can usually be assigned to the class and order to which they belong, even if their systematic position cannot be determined more definitely.

The use of species-pure cultures is a necessary adjunct to the direct examination of sea-water samples when studying the distribution, abundance, and annual fluctuations of nanoplankton, as so little is known of the systematics of these organisms.

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Table VIII. The Comparison of different Speeds and Times of Centrifugation using both 'Normal' Samples and those treated with Potassium Alum ('Floc')*

Sample 1

	'Floc'								'Normal'							
1500 r.p.m. for 10 min. (R.C.F. = 358)																
Total	5	10	12	7	5	6	5	11	5	10	8	8	9	7	5	6
Under 2 μ	..	4	3	3	3	3	3	5	2	5	3	4	4	5	3	4
2–under 5 μ	5	4	3	2	1	2	2	4	1	3	2	3	4	2	2	1
Over 5 μ	..	2	6	2	1	1	..	2	2	2	3	1	1	..	..	1
1500 r.p.m. for 15 min. (R.C.F. = 358)																
Total	7	15	7	11	10	11	12	6	15	11	9	12	8	13	9	5
Under 2 μ	4	8	3	5	4	5	3	3	7	4	2	6	5	5	3	..
2–under 5 μ	2	4	2	3	1	3	5	3	5	4	4	4	2	6	4	4
Over 5 μ	1	3	2	3	5	3	4	..	3	3	3	2	1	2	2	1
1500 r.p.m. for 30 min. (R.C.F. = 358)																
Total	8	11	9	6	11	14	11	10	11	12	14	11	10	6	11	15
Under 2 μ	3	5	4	4	6	4	4	7	6	2	4	3	4	4	3	5
2–under 5 μ	4	5	3	2	4	6	5	3	3	4	5	4	4	2	5	7
Over 5 μ	1	1	2	..	1	4	2	..	2	6	5	4	2	..	3	3
1500 r.p.m. for 1 hr. (R.C.F. = 358)																
Total									12	9	13	12	8	7	6	13
Under 2 μ									5	4	6	6	4	5	2	6
2–under 5 μ									6	4	5	5	3	2	4	5
Over 5 μ									1	1	2	1	1	..	..	2

Sample 2, Knap Buoy, 3. vi. 52

(All counts made with untreated samples)

1500 r.p.m. for 15 min. (R.C.F. = 358)								
Total	24	22	25	29	29	24	26	27
Under 2 μ	13	4	15	18	18	18	17	17
2–under 5 μ	6	8	4	4	9	4	5	5
Over 5 μ	5	10	6	7	2	2	4	5
2000 r.p.m. for 15 min. (R.C.F. = 636)								
Total	25	24	19	30	24	28	24	28
Under 2 μ	16	13	14	19	16	18	13	24
2–under 5 μ	3	3	3	6	4	2	3	2
Over 5 μ	6	8	2	5	4	8	8	2
2000 r.p.m. for 30 min. (R.C.F. = 636)								
Total	24	21	26	23	23	23	33	30
Under 2 μ	12	15	15	17	16	14	22	19
2–under 5 μ	4	4	5	2	4	3	5	7
Over 5 μ	8	2	6	4	3	6	6	4

Sample 3

(Both untreated samples, i.e. 'Normal', and only the totals are given)

1500 r.p.m. for 15 min.	12	14	10	10	12	7	11	7
2000 r.p.m. for 15 min.	11	5	7	10	11	9	9	10

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* Throughout these tables (VIII–XIII), unless otherwise stated, the figures given refer to one field of the Thoma haemacytometer, i.e. they are equivalent to 1 mm.^{3} of the original sample in the case of a centrifuged sample and 5 mm.^{3} of a filtered sample.

TABLE IX. COUNTS FROM THE SAME SAMPLES AFTER CONCENTRATING TO 1/10 WITH THE CENTRIFUGE, AND TO 1/50 WITH THE MEMBRANE FILTER

(All samples are from Knap Buoy)

Sample 1, 27. v. 52																
Centrifuge																
Total	18	16	17	32	27	19	22	24	17	18	16	22	19	17	19	19
Under 2 μ	8	7	9	14	13	9	11	11	6	7	6	10	7	11	10	8
2–under 5 μ	6	7	6	16	12	8	8	10	5	7	7	8	9	5	6	4
Over 5 μ	4	2	2	2	2	2	3	3	6	4	3	4	3	1	3	7
Membrane filter																
Total	130	122	106	88	82	82										
Under 2 μ	78	86	72	54	48	50										
2–under 5 μ	40	30	28	26	26	22										
Over 5 μ	12	6	6	8	8	10										
Sample 2, 29. v. 52																
Centrifuge																
Total	19	15	30	24	24	25	25	19	26	24	22	29	15	22	17	16
Under 2 μ	10	9	14	12	10	13	13	10	13	12	13	14	9	11	8	7
2–under 5 μ	4	3	13	7	10	8	8	6	8	8	8	6	4	7	3	3
Over 5 μ	5	3	3	5	4	4	4	3	5	4	1	9	2	4	6	6
Membrane filter																
Total	63	95	104	90	78	108										
Under 2 μ	40	55	64	60	44	60										
2–under 5 μ	18	30	28	22	24	28										
Over 5 μ	5	10	12	8	10	20										
Sample 3, 3. vi. 52																
Centrifuge																
Total	21	45	39	29	34	37	35	29	41	35	40	31	34	22	25	33
Under 2 μ	11	31	20	18	22	26	23	21	23	23	25	19	21	14	19	21
2–under 5 μ	7	6	12	8	6	7	6	4	10	7	6	6	9	5	2	6
Over 5 μ	3	8	7	3	6	4	6	4	8	5	9	6	4	3	4	6
Membrane filter																
Total	222	186	213	205	180	205										
Under 2 μ	124	110	119	145	130	157										
2–under 5 μ	70	50	60	30	32	24										
Over 5 μ	28	26	34	30	18	24										
Sample 4, 6. vi. 52																
Centrifuge																
Total	31	48	49	32	37	36	25	30								
Under 2 μ	22	36	35	23	25	29	19	18								
2–under 5 μ	4	4	7	2	8	4	4	3								
Over 5 μ	5	8	7	7	4	3	2	9								
Membrane filter																
Total	126	181	191	186	165	188										
Under 2 μ	86	138	124	138	118	140										
2–under 5 μ	26	22	38	26	24	20										
Over 5 μ	14	21	29	22	23	28										
Sample 5, 9. vi. 52																
Centrifuge																
Total	26	39	24	23	24	27	25	33	21	29						
Under 2 μ	16	24	17	16	20	20	16	21	17	23						
2–under 5 μ	7	10	4	4	2	4	6	5	3	2						
Over 5 μ	3	5	3	3	2	3	3	7	1	4						
Membrane filter																
Total	80	169	100	172	161	133										
Under 2 μ	51	130	74	128	124	96										
2–under 5 μ	20	20	18	32	26	26										
Over 5 μ	9	19	8	12	11	11										

TABLE X. TABLE OF COUNTS FROM CENTRIFUGED SAMPLES FROM WHICH SERIAL DILUTIONS WERE ALSO SET UP

Sample 1, 3 miles south of Looe Island, 9. iv. 51

Total	30	39	43	22	39	24	27
Under 2 μ	16	9	8	8	17	12	13
2–under 5 μ	9	11	24	12	11	9	11
Over 5 μ	5	19	11	2	11	3	3

Sample 2, 2. iv. 52

	'Floc'				'Normal'							
Total	9	9	10	8	7	10	9	8	7	10	8	6
Under 2 μ	7	5	7	5	5	5	5	3	5	4	4	3
2–under 5 μ	2	4	3	3	2	4	4	4	2	3	3	2
Over 5 μ	..	..	..	..	...	1	..	1	..	3	1	1

Sample 3, 2 miles south of Rame, 9. iv. 52

	'Floc'								'Normal'							
Total	15	12	10	9	16	8	12	10	9	13	15	9	7	8	7	8
Under 2 μ	7	3	5	6	10	4	7	4	4	6	6	4	2	4	3	3
2–under 5 μ	6	4	3	3	5	4	4	5	2	4	5	4	2	3	3	3
Over 5 μ	2	5	2	..	1	..	1	1	3	3	4	1	3	1	1	2

TABLE XI. TABLE OF COUNTS TO DETERMINE GROWTH-RATES OF CULTURES USED TO SET UP SERIAL DILUTIONS

Days after inoculation	*Pyramimonas* sp.		*Gymnodinium* sp. 2		*Pseudopedinella* sp. 1	
	Mean	σ (theor.)	Mean	σ (theor.)	Mean	σ (theor.)
0	..	..	59	24·3	75	27·4
1	..	..	74	27·2	82	28·6
2	20	14·3	87	29·5	118	34·4
3	36	19·0	110	33·2	131	36·2
4	61	20·47	152	39·0	162	40·3
5	90*	30·0	200*	44·8	180*	42·5
6	..	..	292	54·0	260	51·0
7	400	63·3	..	..	..	..
8	710	84·4	..	..	..	..

	Flagellate K		Flagellate 16	
	Mean	σ (theor.)	Mean	σ (theor.)
0	200	44·7	200	44·7
1	213	46·2	220	47·0
2	...	..	255*	50·5
3	250	50·0	..	..
4	340*	58·4	290	54·0
5	520	72·0	310	55·7

The standard deviation given here is theoretical, based on the Poisson Distribution, and equal to √(Mean of counts).

* Indicates the density of the culture at which the serial dilution was set up. Numbers are given per cubic millimetre of the culture.

Table XII. Counts Obtained from the same Sample after Concentration to 1/10 with the Centrifuge and to 1/63 with the Molecular Filter

(The figures for the counts on the molecular filter refer to one field of the microscope. All samples are from Knap Buoy.)

	Centrifuge										Molecular filter			
Sample 1, 13. xi. 52														
Total	20	17	25	27	19	16	21	16	22	17	132	140	89	97
Under 2 μ	11	10	18	15	12	12	14	9	13	11	112	117	70	78
2–under 5 μ	3	4	5	7	6	3	4	3	4	4	15	15	11	12
Over 5 μ	6	3	2	5	1	1	3	4	5	2	5	8	8	7
Sample 2, 19. xi. 52														
Total	21	19	26	17	13	12	19	23	25	23	117	119	94	96
Under 2 μ	14	13	19	13	9	10	13	16	18	18	100	101	79	80
2–under 5 μ	6	3	5	3	3	1	3	4	4	3	12	15	9	11
Over 5 μ	1	3	2	1	1	1	3	3	3	2	5	3	6	5
Sample 3, 21. xi. 52														
Total	25	24	24	18	25	18	25	15	26	18	149	134	129	135
Under 2 μ	15	12	18	11	19	12	19	10	16	12	121	115	107	115
2–under 5 μ	7	8	5	4	4	4	4	3	6	4	21	13	18	14
Over 5 μ	3	4	1	3	2	2	2	2	4	2	7	6	4	6
Sample 4, 28. xi. 52														
Total	30	33	20	19	29	26	23	20	20	23	149	137	150	144
Under 2 μ	20	17	15	12	22	20	15	14	12	16	111	101	114	105
2–under 5 μ	7	10	3	5	5	5	6	5	6	6	33	26	28	28
Over 5 μ	3	6	2	2	2	1	2	1	2	1	5	10	8	11
Sample 5, 4. xii. 52														
Total	27	25	22	23	24	18	20	26	27	21	163	152	165	143
Under 2 μ	17	18	15	16	16	11	10	18	21	12	138	118	135	115
2–under 5 μ	5	5	4	4	3	5	6	6	4	6	16	24	19	17
Over 5 μ	5	2	3	3	5	2	4	2	2	3	9	10	11	11

TABLE XIII. COUNTS OBTAINED FROM THE SAME SAMPLE BY (*a*) CONCENTRATING TO 1/10 WITH THE CENTRIFUGE AND (*b*) COUNTING UNDER DARK-GROUND ILLUMINATION

(Numbers here are equivalent to 0·1 mm.^{3} of the original sample for a straight count, 1 mm.^{3} for a sample concentrated by centrifuging and 8·66 mm.^{3} in the dark-ground count.)

(*a*) *Counts of cultures*

					Culture diluted: dark-ground counts				
Organisms	Straight count				Motile cells		Motile and non-motile cells		
Chromulina pusilla Butcher	386	480	412		190	206	419	359	
Hemiselmis rufescens Parke	184	170	212	190	243	234	327	268	332

(*b*) *Counts of samples*

	Centrifuge and count										Dark-ground count		
Sample 1, west end of the Breakwater, 10. xii. 52													
Total	16	17	19	19	19	13	20	15	18	20	107	190	124
Under 2 μ	8	9	10	11	12	8	11	10	9	11	65	138	64
2–under 5 μ	5	4	6	3	3	4	6	3	6	5	21	36	39
5–under 10 μ	3	2	3	2	3	0	3	1	3	3	14	11	14
Over 10 μ	0	2	0	3	1	1	0	1	0	1	7	5	7
Sample 2, 11. xii. 52													
Total	20	22	16	17	21	20	17	19	22	19	182	150	
Under 2 μ	13	11	10	8	12	11	8	9	10	9	113	89	
2–under 5 μ	5	6	4	5	6	8	5	7	8	7	40	38	
5–under 10 μ	1	3	2	3	2	0	3	2	3	3	19	17	
Over 10 μ	1	2	0	1	1	1	1	1	1	0	10	6	
Sample 3, aquarium tank water, 12. xii. 52													
Total	8	6	5	7	6	4	8	7	6	7	62	72	
Under 2 μ	5	4	4	5	4	4	4	4	5	4	41	48	
2–under 5 μ	1	1	1	1	2	0	2	2	1	1	12	16	
5–under 10 μ	1	0	0	1	0	0	1	1	0	1	5	6	
Over 10 μ	1	1	0	0	0	0	1	0	0	1	4	2	
Sample 4, Knap Buoy, 15. xii. 52													
Total	12	10	14	9	12	14	10	8	13	12	96	80	
Under 2 μ	10	7	10	6	7	7	6	6	7	8	56	51	
2–under 5 μ	1	2	3	2	2	3	3	2	4	3	21	17	
5–under 10 μ	1	1	0	1	2	3	0	0	2	1	13	7	
Over 10 μ	0	0	1	0	1	1	1	0	0	0	6	5	

	Straight count										Dark-ground count after dilution to 1/10	
Sample 5, mixed culture of diatoms and flagellates												
Total	11	13	14	13	9	10	13	11	12	10	108	124
Under 5 μ	8	10	9	9	6	8	9	6	7	7	70	82
5–under 10 μ	2	3	3	4	2	1	2	3	4	2	29	27
Over 10 μ	1	0	2	0	1	1	2	2	1	1	9	15

THE FEEDING HABITS OF PLAICE POST-LARVAE IN THE SOUTHERN BIGHT

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(Plate I and Text-figs. 1, 2)

INTRODUCTION

The overfishing of valuable plaice grounds in the southern North Sea, has diverted attention from the natural history of the species (*Pleuronectes platessa*) towards a better understanding of its population dynamics. The early statistical studies of Buchanan-Wollaston (1914–26) have been followed by the recent work of my colleague Mr A. C. Simpson, at the Fisheries Laboratory, Lowestoft, on egg production and survival of larvae over the plaice spawning ground in the Southern Bight.

Observations of a purely biological nature are often needed to clarify the pattern of population fluctuations derived from statistical data. For instance, efforts to assess the mortality of plaice in the Southern Bight had been somewhat impeded by gaps in our knowledge of the food and feeding behaviour of the post-larval phase. In 1950 I was given the opportunity to fill in these gaps.

A survey of literature on this subject revealed a variation in food preferences of post-larvae from different coastal areas of Great Britain. Kyle (1898), in his investigation of the Scottish plaice fishery, discovered young annelid larvae, crustacean ova, *Evadne nordmanni*, and larval molluscs in the post-larval stomachs. Lebour (1919), working at Plymouth, concluded that certain copepods and other Entomostraca formed the main food of nearly all young flatfish caught, though the plaice was poorly represented in this examination. Scott (1922), in the Irish Sea area, found harpacticoids, small copepods and algal spores to be the chief food constituents of the plaice stomachs.

As a result of the dissection of some hundreds of specimens taken in four different years the diet of plaice post-larvae from the Southern Bight is now known, in normal seasons, to be markedly different from that in the areas examined by earlier workers (Table I).

COLLECTION AND DISSECTION METHODS

In the early months of 1946 and 1947, the Hensen net was used to catch planktonic plaice post-larvae during egg-sampling cruises. The Heligoland larva net and the Agassiz trawl were introduced in 1950 and 1951, as work progressed on the post-hatching stages. Larvae and post-larvae were preserved in 2% formalin, to be later measured and classified into developmental stages 1–5. Stage 1 is the larva in which the yolk-sac is not yet resorbed. Post-larval stages 2 and 3 are planktonic and still symmetrical, whereas stage 4 covers the movement of the left eye on to the edge of the head. Stage 5, with both eyes on the right side, is thought to be the first wholely bottom stage, approaching completion of metamorphosis.

TABLE I. SUMMARY OF PLAICE POST-LARVAL FEEDING

Date of cruise	No. of post-larvae examined	Empty stomachs	Post-larvae feeding on *Oikopleura*	% feeding post-larvae containing *Oikopleura*	Other food
1946					
20–22 Jan.	158	80	65	82·5	Few copepod nauplii, significant numbers *Coscinodisus* spp., green food remains
1947					
22–28 Jan.	1	—	1	—	Nil
13–18 Feb.	35	11	—	—	Copepod nauplii, invertebrate eggs, bivalve veliger, *Coscinodiscus* frustules, green food remains
11–14 Mar.	19	7	—	—	Copepod nauplii, copepodites, small copepods, diatom debris
22–28 Mar.	6	1	—	—	*Temora longicornis* nauplii and polychaete bristles
1950					
9–12 Mar.	83	23	47	78·3	2 *Temora longicornis*, 3 copepod fragments, 1 polychaete larva, occasional diatom frustule
1951					
10–20 Feb.	32	17	12	80·0	Only few diatom frustules
27 Feb.–7 Mar.	34	14	19	95·0	1 gastropod veliger, few diatom frustules
20–31 Mar.	129	65	51	79·7	2 *Temora longicornis*, 3 fragmentary copepods, 5 with palps of *Magelona papillicornis*, occasional copepod eggs, single gastropod veliger

In 1950 and subsequent years, the gut contents were examined as follows. Specimens were placed on a black background, in order to emphasize the opaque outlines of the coiled alimentary canal. The body wall was dissected away on one side with needles; the gut severed in the anterior oesophageal and posterior anal regions, then removed into water in a watch-glass. No optical aids were needed. After disengaging liver and muscular tissue under a dissecting microscope, the gut was transferred to a drop of water on a slide and divided into three sections, by transverse cuts at the anterior and posterior stomach sphincters. These sections were laid open with needles, the lumen contents separated from gut tissue by gentle teasing, and the former examined under high power.

THE DIET IN RECENT YEARS

In February–March 1950, eighty-three undamaged post-larvae, between 5·5 and 15·5 mm. in length, were taken in the Agassiz trawl off the Dutch coast. Subsequent examination of the gut contents showed that forty-seven specimens contained black cigar-shaped pellets, always associated with transparent, indigestible matrix vesicles, arranged along the length of the gut like peas in a pod (Plate I, fig. 1). Only five specimens held the chitinous remains of copepods; in two these remains were identified as *Temora longicornis*, in the other three they were too fragmentary for identification. Twenty-three stomachs were empty. Diatoms were usually found associated with animal food remains, the principal genera being *Coscinodiscus*, *Biddulphia*, *Navicula* and *Nitzschia*. The larger thin-walled diatoms are readily taken by the yolk-sac stage larvae and the early stage-2 post-larvae. They probably represent the 'green food remains' observed in the past by a number of workers.

The black pellets and surrounding matrix vesicles were studied more closely (Pl. I, fig. 2). Pellets varied greatly in length (0·12–0·60 mm.), but their shape was more consistent. Their number varied from specimen to specimen, and their distribution within the gut was not constant. As many as sixty-two pellets were found in one gut alone, forty-two in the stomach and twenty in the intestine. As fish post-larvae are not known to produce compact faeces, these pellets were judged to be formed by a certain type of animal prey. The prey would have been soft, easily and almost completely digestible, leaving characteristically shaped pellets and a thin matrix vesicle as the only evidence of its nature. Zooplankton samples from the spawning area were examined for such an animal. *Oikopleura dioica* (Pl. I, fig. 4) fitted the description and was present at all stations where post-larvae occurred. The shape variation of pellets from the gut of *Oikopleura* and from the gut of post-larvae taken in the same locality are compared in Text-fig. 1. There is a distinct relationship. The indigestible matrix vesicles were, without doubt, the developing 'houses' of *Oikopleura* eaten by the young plaice. Lohmann (1903), in his work on the Appendicularia, noted the resistant nature of these structures. The cuticular secretions of the tunicates are now known to have a chemical composition closely allied to cellulose. The absence of cellulase from the enzymic system of the plaice post-larva would explain the indigestibility of the 'house'.

During the spring of 1951, a further series of Agassiz hauls was made over the plaice spawning grounds. A total of 195 post-larvae were caught, eleven of which bore undigested *Oikopleura* in the oesophagus (Pl. I, fig. 3), whilst a further seventy-one contained typical pellets and vesicles. Seventeen specimens held other animal food, principally *Temora longicornis*, copepod eggs and molluscan veligers. The palps of *Magelona papillicornis*, a sedentary polychaete, were found in the stomachs of a few late stage-5 post-larvae.

TABLE II. ABSTRACT OF PLANKTON ANALYSIS FOR THE SOUTHERN BIGHT, 1947

Main potential food organisms of plaice post-larvae per cubic metre. Vertical Hensen net hauls (60 meshes/1 in.).

	Station no.							
Species	1	2	3	4	5	6	7	8
	Month of cruise: January							
Small copepods	N.D.	87	205	234	214	445	868	N.D.
Polychaete metatrochophores	N.D.	—	—	—	—	—	—	N.D.
Polychaete post-larvae	N.D.	—	—	—	—	—	20	N.D.
Lamellibranch post-larvae	N.D.	4	—	21	164	70	39	N.D.
Oikopleura spp.	N.D.	4	—	9	7	3	20	N.D.
Coscinodiscus concinnus	N.D.	—	—	—	33	127	296	N.D.
Biddulphia regia	N.D.	—	—	47	296	19,826	26,250	N.D.
	Month of cruise: March							
Small copepods	103	56	77	77	3,796	345	329	129
Polychaete metatrochophores	4	9	—	—	56	—	16	4
Polychaete post-larvae	—	—	—	—	—	—	—	—
Lamellibranch post-larvae	—	—	22	—	422	—	41	—
Oikopleura spp.	—	—	11	—	—	—	—	—
Coscinodiscus concinnus	123	59	33	131	12,656	181	1,376	16,286
Biddulphia regia	—	2	153	1,796	1,026,563	3,055	7,904	129
	Month of cruise: April							
Small copepods	1	131	112	136	129	173	94	92
Polychaete metatrochophores	—	6	13	—	—	12	23	—
Polychaete post-larvae	—	—	—	14	—	—	—	26
Lamellibranch post-larvae	—	—	—	—	—	—	—	—
Oikopleura spp.	—	—	—	—	—	—	—	—
Coscinodiscus concinnus	59	938	108	32	246	190	598	99
Biddulphia regia	—	47	15,361	289	43	138	105	59
	Month of cruise: May							
Small copepods	2,918	9	11	82	195	664	1,059	N.D.
Polychaete metatrochophores	14	—	—	—	—	—	4	N.D.
Polychaete post-larvae	—	—	—	3	—	—	20	N.D.
Lamellibranch post-larvae	—	—	—	—	—	—	16	N.D.
Oikopleura spp.	—	—	—	—	—	—	—	N.D.
Coscinodiscus concinnus	879	104	203	—	3	—	4	N.D.
Biddulphia regia	8	66	53	—	25	—	—	N.D.

N.D. = no data.

Station nos. 1–8 represent a line of stations 12 miles apart stretching across the Southern Bight, from Smith's Knoll to the Hook of Holland.

TABLE III. ABSTRACT OF PLANKTON ANALYSIS FOR SOUTHERN BIGHT, EARLY MARCH 1950

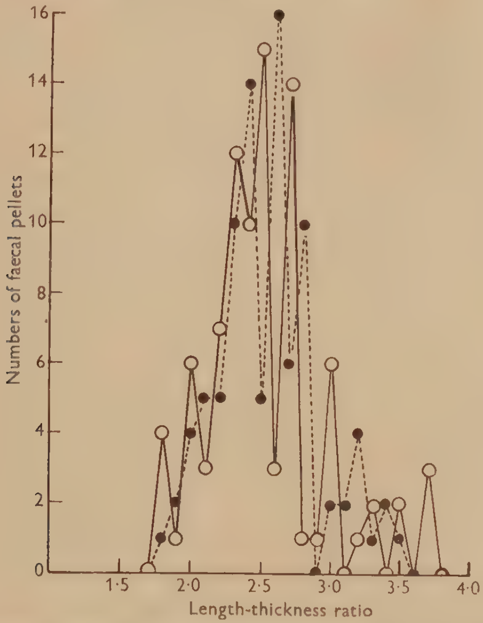
Main potential food organisms of plaice post-larvae per m.^{3}. Vertical Hensen net hauls (60 meshes/1 in.).

	Station no.									
Species	11	12	23	24	25	34	35	38	39	48
Small copepods	951	1,987	191	613	239	234	244	285	204	40
Lamellibranch post-larvae	—	—	6	5	7	—	3	—	—	2
Oikopleura spp.	82	52	181	—	83	6	54	7	41	13
Coscinodiscus concinnus	—	—	—	—	—	—	—	—	—	10
Biddulphia regia	74,320	142,629	2,694	2,885	128,892	89	7,398	152	38	31

Plankton organisms less than 0·35 mm. in length are not filtered quantitatively by the Hensen net. However, size measurements of the faecal pellets taken from plaice stomachs suggest that smaller *Oikopleura* were either relatively scarce or were not used as food by plaice post-larvae.

Ninety-six stomachs were empty. Towards the end of March 1951, fifty completely metamorphosed young plaice (15–17 mm. long), collected at stations about 25 miles west of Ijmuiden, were found to be feeding mainly on the palps of *M. papillicornis*.

Records for January 1946 show that a high proportion of feeding post-larvae of an early stage, had 'detrital pellets' in the alimentary canal. On the other hand, post-larvae caught with a Hensen net during 1947 appear to have fed on a mixed diet of large diatoms and small copepods, with an occasional molluscan veliger and polychaete larva.



Text-fig. 1. Variation in length/thickness ratio of equal numbers of pellets, from the gut of plaice post-larvae (black circles—continuous line) and of *Oikopleura dioica* (open circles—broken line).

Thus in 1946, 1950 and 1951, plaice post-larvae from the Southern Bight seem to have utilized the small population of *Oikopleura* present as a principal source of animal food during their planktonic life (Table I). Preliminary observations for 1952 give a similar picture.

The anomalous situation in early 1947, when other species assumed principal importance as food, has yet to be explained. Probably the prolonged easterly gales and icy conditions in the plaice spawning area delayed the annual increase of *Oikopleura* stocks. The organisms comprising this alternative diet are usually more abundant than *Oikopleura* in the plankton during the post-larval development period of the plaice (Tables II and III).

THE PHENOMENON OF FOOD SELECTION

There is much evidence to support the view that flat-fish post-larvae are discriminate feeders. Dannevig (1897) was of the opinion that only one food species was eaten for a time by baby plaice, though different individuals may eat different species. Petersen (1894) observed that dabs, plaice and flounders living under the same food conditions, had distinctly different food preferences. Lebour (1919) concluded that most young fish select their food from what is commonest in the plankton at the time, indiscriminate feeding seldom or never taking place.

In the present work, the food eaten by plaice post-larvae bore little relationship to the proportions of the plankton constituents. As an example, the summary of feeding during March 1950 (Table I), should be compared with the analysis of plankton taken in the same area at the same time (Table III). Feeding was restricted to a few species, and was not in proportion to the abundance of those species in the plankton.

The mechanism of diet restriction is still in doubt. The popular term 'food selection' has in the past been used to describe an effect, with the implication that fish post-larvae instinctively select certain food species when presented with a choice. There is no question of post-larvae being inherently fitted to utilize only a limited number of the species of prey normally available to them in their natural environment. North Sea plaice post-larvae have been successfully reared in this laboratory on *Artemia salina* nauplii, which they do not encounter in nature. In 1951, stage-2 post-larvae fed eagerly on dried egg fragments sprinkled over the surface of sea water in tanks.

Conceivably, under normal circumstances, young fish are able to recognize suitable food after a period of exploratory hunting activity, and the extent of this suitability may be largely governed by the vulnerability of the prey. Vulnerability would seem to depend on the size, structure and behaviour of the prey, as well as on the hunting ability of the predator.

The importance of prey size in relation to the mouth and gullet proportions of a post-larva was discussed by Lebour (1919), and elaborated recently by Wiborg (1948) for cod larvae. Observations made at sea in March 1952 revealed that stage-3 plaice post-larvae were feeding on *Oikopleura*, *Temora* nauplii, metanauplii and copepodites, which were particularly abundant, and on the large diatoms *Coscinodiscus concinnus* and *Biddulphia regia*. These organisms were occasionally found together in the same stomach. Post-larvae in earlier stages of development were eating smaller *Oikopleura*, nauplii and the usual diatoms. Advanced larval and early post-larval forms fed on diatoms only, the smallest of these food types.

Structure and behaviour play an equally important part in determining the vulnerability of prey. Lebour (1918) noticed that decapod larvae were rarely eaten by young fish, and attributed this to the spinous nature of the prey.

The number and efficiency of sense organs, conspicuousness, special escape reactions, secretion of repellents, and the swarming habit, are but a few of the factors which might well decide how far a particular prey is open to attack by a particular predator.

With the concept of vulnerability in mind, it is easy to see why the soft-bodied, slow-moving, but conspicuously vibratile *Oikopleura* should fall a ready victim to a visual feeder, as the plaice post-larva appears to be (see below).

DIURNAL FEEDING ACTIVITY

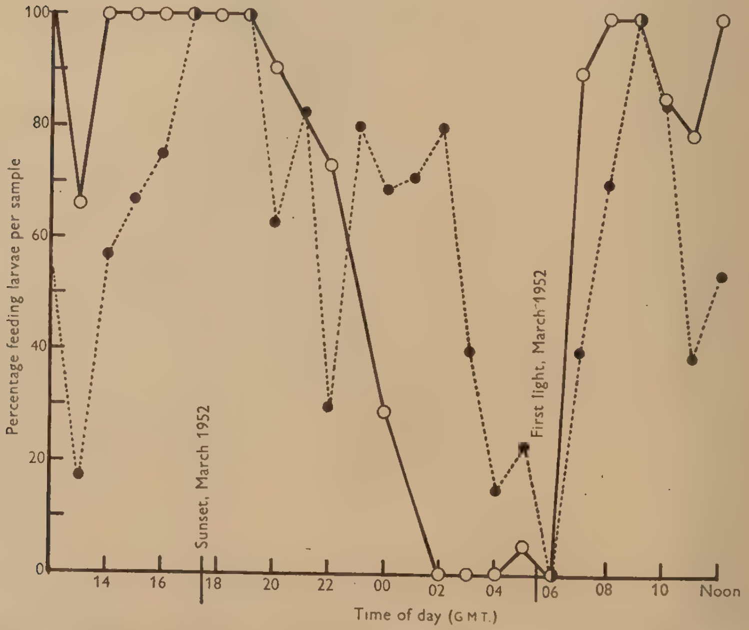
Forty-four per cent of all post-larval stomachs were empty. Other workers have referred to the lack of food traces in a high proportion of the total stomachs they investigated. Scott (1922) thought that the easy rupture of the stomachs might account for emptiness in the early planktonic forms, and that regurgitation in the net, or on addition of preservative, took place with metamorphosing and later stages. Lebour (1921) commented on the high numbers of empty stomachs encountered during work at Plymouth, and considered that infrequent feeding coupled with rapid digestion, might serve as a protective measure in young clupeoids. In these circumstances, feeding fish would only occasionally be caught.

The regurgitation theory was tested at sea in March 1952. Two vertical Heligoland larva-net hauls were made every 2 hr. in 20 fathoms of water. The plaice larvae and post-larvae of each haul were separated from other planktonic material. One catch was dissected immediately for stomach contents, and the other preserved in 2% formalin for later dissection. After 24 hr. work, no significant difference was seen in the frequency of empty stomachs between the two samples from any 2-hourly station, and the conclusion was reached that the addition of dilute formalin did not stimulate antiperistalsis. However, as the experiment progressed from day to night, a distinct falling off in the proportion of filled stomachs was noticed, followed by a sudden burst of feeding activity after first light the following morning. The experiment was continued for a further 24 hr. The pooled results for both days are represented in Fig. 6 by the continuous line. Post-larval feeding was most intense shortly after first light and for some considerable time before darkness fell, with a period of desultory feeding before and after noon. Data for the 4 years under consideration in this paper were analysed in a similar way to those of the 48 hr. station, and the results are presented in Table IV and Text-fig. 2 by the broken line. The principal gradients are not as sharply defined as for the 48 hr. station, due to differences in the extent of daylight hours between January and late March, but the first light and afternoon peaks of feeding activity are quite distinct, with a decline occurring in the early hours of the morning.

Light conditions seem to play an important part in determining the

TABLE IV. INCIDENCE OF PLAICE POST-LARVAE WITH FOOD IN THE STOMACH

	48 hr. station		Other stations	
Hour	Total larvae	No. feeding	Total larvae	No. feeding
13.00	6	4	12	2
14.00	11	11	7	4
15.00	4	4	9	6
16.00	16	16	28	21
17.00	1	1	8	8
18.00	10	10	—	—
19.00	4	4	9	9
20.00	37	34	40	25
21.00	—	—	41	34
22.00	46	35	70	21
23.00	—	—	15	12
Midnight	14	4	16	11
01.00	—	—	35	25
02.00	3	0	20	16
03.00	7	0	20	8
04.00	19	0	13	2
05.00	19	1	17	4
06.00	24	0	2	0
07.00	51	46	15	6
08.00	9	9	13	9
09.00	19	18	2	2
10.00	7	6	13	11
11.00	8	7	26	10
Noon	12	12	67	36



Text-fig. 2. Feeding periodicity of plaice post-larvae. Percentage of larvae in each sample with food in the gut, from the 48 hr. station, March 1952 (open circles—continuous line), and from other stations of recent years (black circles—dotted line).

periodicity of feeding activity in plaice post-larvae. This conclusion is reinforced by laboratory work, in which the post-larvae of plaice and plaice-flounder hybrids were subjected to changes in light conditions, other environmental factors being equal. The experiment was carried out at 9° C. using *Artemia salina* nauplii as food. On the first day of the experiment, five beakers containing batches of post-larvae were standing in tank *A*, surrounded by circulating water at 9° C., open to artificial and diffuse daylight. That same night, three of these beakers (7, 12 and 15*a*) were transferred to tank *B*, in which conditions were the same as in tank *A*, except for the complete exclusion of light. These three beakers were returned to tank *A* the following evening, whilst the remaining two beakers (11 and 15*b*) took their place in darkened tank *B*. On the evening of the third day, beakers 11 and 15*b* were transferred back to tank *A*. Observations on post-larval feeding activity were made each day at approximately 10.30, 15.00 and 18.00 hr., and the results are recorded in Table V. On no occasion were post-larvae found feeding in darkness; in lighted conditions plaice-flounder hybrids were more vigorous feeders than plaice.

TABLE V. THE FEEDING ACTIVITY OF POST-LARVAE IN RELATION TO LIGHT

1952	Time	Plaice 7	Plaice 11	Hybrid 12	Hybrid 15*a*	Hybrid 16*b*
29 Jan.	11.00	Light 1/7	Light 1/6	Light 9/9	Light 4/10	Light 1/10
	15.20	Light —	Light —	Light —	Light —	Light 7/10
	17.30	Light 2/7	Light 3/6	Light 8/9	Light 7/10	Light 7/10
30 Jan.	11.00	Dark 0/7	Light 2/6	Dark 0/9	Dark 0/10	Light —
	13.00	Dark 0/7	Light —	Dark 0/9	Dark 0/10	Light 9/10
	15.15	Dark 0/7	Light 3/6	Dark 0/9	Dark 0/10	Light 8/10
	18.00	Dark 0/7	Light 4/6	Dark 0/9	Dark 0/10	Light 8/10
31 Jan.	10.30	Light 1/7	Dark 0/6	Light 5/9	Light 6/10	Dark 0/10
	14.30	Light 5/7	Dark 0/6	Light 7/9	Light 8/10	Dark 0/10
	18.00	Light 7/7	Dark 0/6	Light 7/9	Light 8/10	Dark 0/10
1 Feb.	10.40	Light 2/7	Light 0/6	Light 4/9	Light 6/10	Light 8/10
	18.00	Light 4/7	Light 2/6	Light 7/9	Light 4/10	Light 9/10

The fractions give the number of post-larvae with food in their stomachs (numerator) and the total number of post-larvae in the beaker (denominator).

Thus there are good grounds for concluding that plaice post-larvae are visual feeders, although the possibility of an inherent daily feeding rhythm cannot be entirely overlooked. For present purposes, it is sufficient to say that a large proportion of the empty stomachs encountered during the investigation of their diet in the Southern Bight, can be accounted for by big diurnal fluctuations in feeding activity influenced by light conditions.

I am indebted to members of the staff of the Fisheries Laboratory at Lowestoft, in particular to Mr A. C. Simpson, for the experimental data on the feeding periodicity of post-larvae in tanks and for access to material from his plaice and larva surveys of 1946–52. I would also like to thank Mr J. C. Cattley for the plankton analyses and Dr H. A. Cole of the Shellfish Experimental Station, Conway, for his criticism of the manuscript.

SUMMARY

An examination was made of the stomach contents of plaice post-larvae taken from the Southern Bight spawning ground in the spring of 1950. They were found to be feeding mainly on a soft-bodied prey, which left characteristically shaped faecal pellets and an undigested matrix vesicle as the only evidence of its nature. Pellets from the post-larvae compared closely in shape with those of *Oikopleura dioica*, found in the plankton of the spawning area during the period of post-larval development.

In 1951, undigested *Oikopleura* were seen in the oesophageal regions of a few dissected specimens, in addition to typical indigestible remains in a large proportion of the total feeding post-larvae examined.

Records for 1946 suggest a similar exploitation of the *Oikopleura* population by plaice post-larvae, although the phenomenon was not repeated in 1947, when inclement weather may have interfered with the annual outburst of this prey.

The phenomenon of food selection by plaice post-larvae is discussed, with special reference to the part that vulnerability may play in limiting the number of species of prey available as food.

A feature of this and other investigations on post-larval feeding, was the occurrence of a high proportion of empty stomachs. An experiment designed to test a regurgitation hypothesis, revealed the existence of a marked diurnal pattern of feeding activity, apparently influenced by light conditions. The importance of light in feeding behaviour was confirmed by tank experiments. In this investigation a large proportion of the empty stomachs encountered can be explained by reference to diurnal fluctuations in feeding activity, supporting the view that plaice post-larvae are visual feeders.

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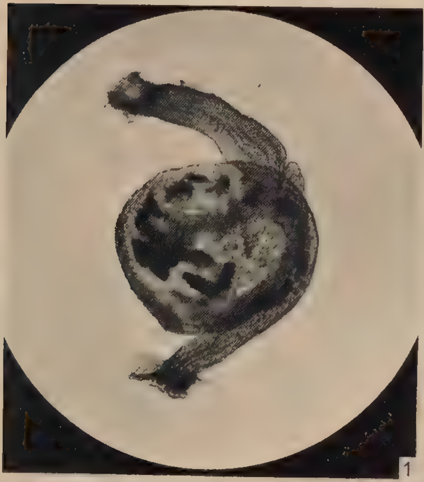
EXPLANATION OF PLATE I

Fig. 1. Complete gut of plaice post-larva, showing large pellets and matrix vesicles *in situ*; × 40.

Fig. 2. Small pellets and vesicles after removal of the gut; × 50.

Fig. 3. Undigested *Oikopleura dioica* (left centre) removed from the oesophagus (left), and matrix vesicles (right) from the stomach; × 50.

Fig. 4. Selected *Oikopleura dioica* from plankton sample. Southern Bight, February 1951; × 50.



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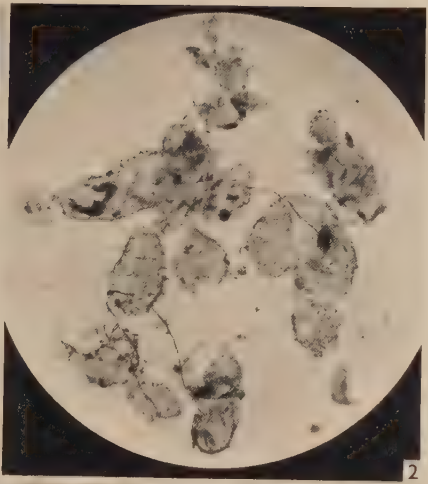

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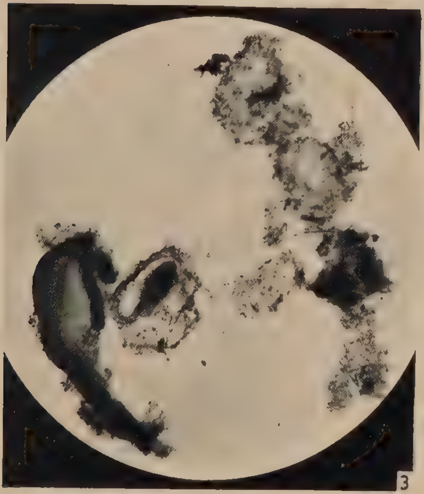

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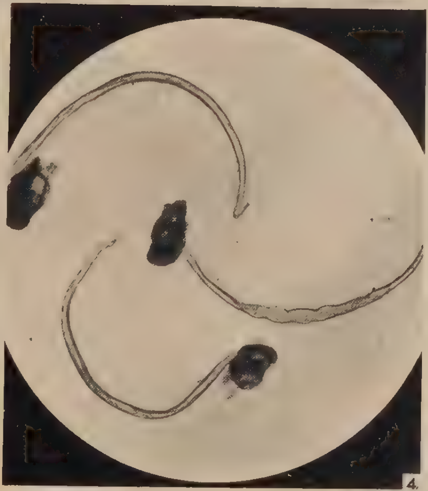

4

STUDIES IN THE PHYSIOLOGY OF COMMENSALISM. III. THE POLYNOID GENERA *ACHOLOË*, *GATTYANA* AND *LEPIDASTHENIA*

By Demorest Davenport

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(Text-fig. 1)

The following investigations were conducted at the Plymouth Laboratory under a grant from the John Simon Guggenheim Memorial Foundation. The author wishes to express his appreciation to the Foundation for its support; to the Director and members of the staff of the Plymouth Laboratory for their friendly co-operation and assistance; and in particular to Dr D. P. Wilson for his continued interest and aid.

INTRODUCTION

Previous experiments (Davenport, 1950; Davenport & Hickok, 1951) gave evidence that in certain echinoderm-polynoid partnerships the specificity in part depended upon a positive response of the commensals to a substance or substances released by their hosts. In the partnership between the starfish *Evasterias troschelii* Stimpson and the polynoid *Arctonoë fragilis* (Baird) the attractant was found to be non-dialysable and relatively unstable; but, as demonstrated by the Y-tube choice-apparatus employed, enough attractant was continuously released by the host to affect the activity of the commensal at a considerable distance.

Experiments with the scale-worm *Halosydna brevisetosa* Kinberg, commensal with the terebellid *Amphitrite robusta* (Johnson), gave no evidence that a chemical attraction plays an important part in governing this association, at least when the partners are adult. However, a single unpublished experiment conducted during the summer of 1952 at the Kerckhoff Marine Laboratory in California showed that the commensal *Hesperonoë adventor*, which lives in the tube of the echiuroid *Urechis caupo*, is effectively 'tied' to its host with a powerful bond. An *Hesperonoë* can unerringly distinguish water in which its host is housed from plain sea water, and will make an active search for its host when separated from it. In this strict association the polynoid is perhaps conditioned to whatever substance it is that gives *Urechis* its familiar, powerful, aromatic smell.

The present work constitutes an effort to elucidate the mechanism of control of additional commensal partnerships.

ACHOLOË ASTERICOLA (DELLE CHIAJE)

Material and Methods

At Plymouth the partnership between the starfish *Astropecten irregularis* (Pennant) and the polynoid *Acholoë astericola* (Delle Chiaje) is well known (Fig. 1), and the animals are readily available for investigation. In collections regularly brought into the Laboratory from the Eddystone Grounds, at times as many as 75% of the starfish have the small orange polychaete in the ambulacral groove. Ordinarily only one worm per starfish is found, but very occasionally two are present, which may be markedly different in size and age.

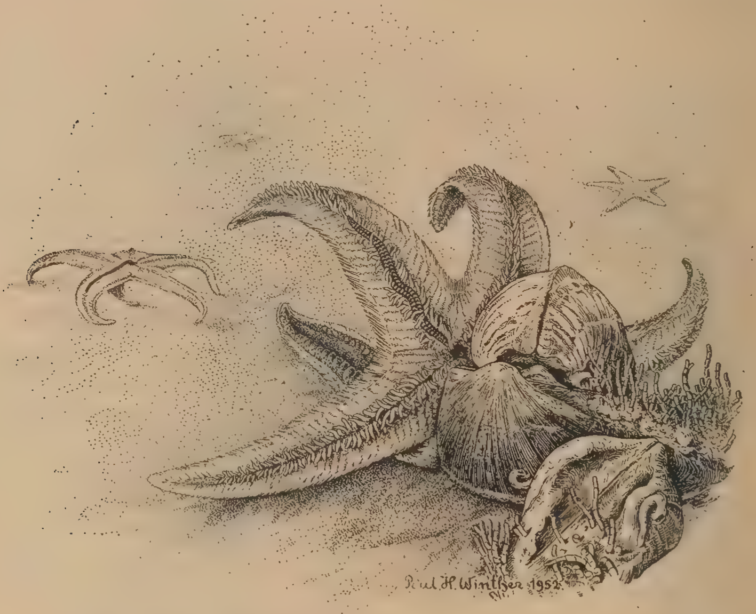

Fig. 1. The host starfish *Astropecten irregularis* showing the commensal *Acholoë astericola* in the ambulacral groove with head directed towards the stomach of the host, into which it may extend when feeding.
(By Courtesy of Gunnar Thorson, from studies made in Dakar, Senegal, April 1952. Drawn by Poul H. Winther.)

Astropecten is the only known host of *Acholoë* at Plymouth (Marine Biological Association, 1931). In the Mediterranean the polynoid occurs on *Astropecten aurantiacus* and *A. pentacanthus* (McIntosh, 1900). Panceri (1874) records the occurrence of *Acholoë* on the starfish *Luidia ciliaris*

(Philippi) (=*L. fragilissima* Forbes) in the Mediterranean, but no such association is known in Plymouth waters, in spite of the fact that at times *Luidia* is relatively common in the same environment as commensal-carrying *Astropecten.*

At the outset it was obviously important to determine whether the commensal made any constant response to the host. As observed by many workers, if the *Acholoë* were removed from the host and the tip of the arm of the host placed in close proximity to the head, the worms almost immediately became activated and fastened themselves to the host, either climbing quickly on to the aboral surface, or into the ambulacral groove, or wrapping themselves entirely around the tip of the host's arm. Brief experiments indicated that single tube-feet removed from the host would elicit a typical response. If a tube-foot was presented to its head, the *Acholoë* became active and with a twisting motion quickly wrapped itself around the tube-foot and the tip of the holding forceps. This response could only be elicited by contact. In many experiments tube-feet were held within a millimetre or less of the head of the commensal without eliciting any reaction. In addition, several experiments demonstrated that a small quantity of non-circulating sea water which had surrounded two host starfishes in a 200-ml. beaker for 2 hr. had no effect whatever on the behaviour of the commensals when brought near the head by a fine pipette. However, under normal conditions contact of host tissue with the head of the commensal 'triggered' an immediate and typical reaction.

The facility with which this typical and constant response can be evoked from a random sample of worms, and easily manipulatable materials can be presented while the commensals are under observation, has made the partnership particularly productive for investigating the problem of specificity and the nature, source and effect of the attractant.

In the following experiments standard procedure was to isolate six *Acholoë*, selected at random, in shallow dishes of fresh 'outside' sea water. Responses were elicited by presenting materials held in the tip of fine-pointed forceps. An effort was made to keep all materials in one series of tests of approximately the same size. In each experiment materials were presented in rapid order to the six worms and the responses recorded. Between tests of each material the commensals were washed in clean 'outside' water and instruments in alcohol-acetone.

Responses were recorded as follows. If on presentation of the test material the commensal was immediately activated and responded by quickly moving on to or wrapping itself around the material, the response was recorded as +. If a response appeared only after the material had been in contact with the head for several seconds or if the activity produced was sluggish, this was recorded as (+). No response whatever, after insistent presentation, was recorded as 0.

Observations

Experiment No. 1. Is the response specific? Single tube-feet from members of the genera of starfish represented in Plymouth waters were presented; and reactions recorded in Table I.

As can be seen, the response is only relatively specific. The majority of *Acholoë* behave consistently in the presence of each starfish presented, but an occasional individual (e.g. A, 3) seems unable to 'tell the difference', a response being elicited by the tube-foot of any starfish.

TABLE IA

Worm	*Astropecten irregularis* (host)	*Asterias rubens*	*Marthasterias glacialis*	*Luidia ciliaris*	*Porania pulvillus*
1	+	o	o	+	(+)
2	+	o	o	(+)	(+)
3	+	+	(+)	+	(+)
4	+	o	o	+	(+)
5	+	o	o	+	o
6	+	o	o	(+)	o

TABLE IB

Worm	Host control	*Palmipes membranaceus*	*Solaster papposus*	*Henricia sanguinolenta**	*Stichastrella rosea**	*Asterina gibbosa**
1	+	(+)	o	(+)	o	(+)
2	+	(+)	o	(+)	o	(+)
3	+	(+)	(+)	(+)	o	(+)
4	+	(+)	o	(+)	o	(+)
5	+	(+)	o	(+)	o	(+)
6	+	(+)	o	(+)	o	(+)

* Small size of tube-feet necessitated presentation of tip of arm of starfish.

Unfortunately, because of the limited availability of experimental animals, experiments could not be conducted with a large enough sample of commensals to give a statistically significant quantitative evaluation of the difference of intensity of response to each form. However, from the above data certain conclusions can be drawn.

It can be seen that *Acholoë* respond immediately and with highest intensity to host tube-foot, and with about equal intensity to the tube-feet of *Luidia*. This is of particular interest in view of the absence of *Acholoë* on the *Luidia* of Plymouth waters, where *Luidia* may be taken in the same dredge-haul with *Astropecten*. Curiously, the above-cited reference to the occurrence of *Acholoë* on *Luidia* in the Mediterranean was not found in the literature until after the above experiments had been completed. At the time of writing, *Acholoë* placed on a large *Luidia ciliaris* have lived 5 months in the 'Drake's Island' Tank at Plymouth. The worms do not appear to take up position in the ambulacral grooves, but cling to the sides and aboral surface of the unaccustomed host.

The above data also indicate that the *Acholoë* respond to some extent to all members of the asteroid order Phanerozonia available at Plymouth (*Astro-*

pecten, *Luidia*, *Porania*), to certain Spinulosa (*Asterina*, *Palmipes*, *Henricia*), but not ordinarily to Forcipulata (*Asterias*, *Marthasterias*, *Stichastrella*). This perhaps indicates some biochemical similarity among these members of the Phanerozonia and Spinulosa. Evidently the starfish *Porania*, *Palmipes*, *Asterina* and *Henricia*, which elicited a delayed or weak response, either produce a smaller amount of attractant or one slightly different from that of the host or *Luidia*. At any rate their attraction is enough to cause the commensals to become attached to them when contact occurs. The answer to the strict specificity of *Acholoë* and *Astropecten* in Plymouth waters, in view of the attraction which a number of starfish show for this polynoid, can only be sought in further laboratory and field observations.

Experiment No. 2. What can be determined concerning the source of the attractant in the host? Small pieces of host tissue which had been thoroughly washed in clean sea water were presented, with the following results:

Worm	Spine	Aboral integument	Stomach	Gastric caecum	Tube-foot control
1	+	+	+	0	+
2	+	+	+	+	+
3	+	+	+	+	+
4	(+)	+	+	(+)	+
5	+	+	+	+	+
6	+	(+)	+	(+)	+

These clearly show that all parts tested had some attraction for commensals. Again, the sample of worms available for testing was not large enough for a quantitative evaluation of the difference in intensity of attraction of each tissue. It appeared that stomach triggered off the most active and enthusiastic response, but this may have been the result of using slightly larger masses of stomach. However, because of the ready response elicited, a number of subsequent experiments have been conducted with stomach as the source of attractant.

These data also offer rather persuasive evidence that texture or consistency of surface, in which the tissues differed widely, have little to do with the response.

Experiment No. 3. The question was now asked whether the attractant produced by the stomach is released into the stomach cavity, and whether a response can be elicited by the presentation of material that has been soaked in stomach contents.

Small pieces of cotton-wool were introduced into the stomachs of six *Astropecten* and left for 1 hr. They were then presented to six *Acholoë*, whose reactions were:

Worm	Test cotton-wool from stomach	Control cotton-wool	Control stomach
1	(+)	0	+
2	0	0	0
3	+	0	+
4	+	0	+
5	+	0	+
6	(+)	0	(+)

Thus apparently attractant is present in stomach contents. That attractant absorbed on cotton-wool will elicit a response presents additional evidence that surface texture plays little part in the response and provides a tool with which it is possible to investigate the presence of attractant in extracts, etc.

The above results would scarcely have been obtained had there not been a quantity of attractant in the stomach contents. That not enough attractant diffuses from the general surface of the host to affect the behaviour of the commensals at a distance has already been noted. Attractant may collect in the stomach to give a higher concentration than occurs in the water immediately surrounding the host, or in the stomach the attractant may be protected (from oxidation?).

No indication has been given, however, that attractant diffuses from isolated stomach more readily than from other tissues. Although, as noted above, stomach elicits a strong response from commensals, contact still appears to be necessary. This was indicated in the following experiment.

Experiment No. 4. A small bag of 100-mesh bolting-silk was firmly filled with isolated stomach. The bag was presented.

Worm	Bag of stomach	Control stomach	Control bag
1	(+)	+	0
2	0	+	0
3	(+)	+	0
4	(+)	+	0
5	(+)	+	0
6	0	+	0

The results clearly show that when bolting-silk separates the head of the worm from stomach tissue the response is markedly weakened. The weakened responses elicited from four of the worms may have been the result of contact with small bits of stomach protruding through the holes in the silk. One set of experiments has already shown the unimportance of surface texture, and here the failure of the normal response appears to be due to the separation of the head by the bolting silk from the surface of the material to which attractant is closely bound.

These experiments indicate that for normal responses to be elicited contact with a surface to which the attractant appears to be closely bound is necessary.

The effects were then tested of certain physical factors (time duration, temperature change) on the attracting ability of whole isolated host tissue.

Experiment No. 5. Does the passage of some hours decrease the ability of isolated tissue to attract, provided it stays fresh? Fresh stomach pieces elicited a positive response from six commensals. After the passage of 18 hr. in clean sea water at 15° C. the pieces were as effective as ever.

Experiment No. 6. Can a response be elicited to refrigerated whole host tissue? Pieces of stomach were placed in 300 ml. of clean sea water, refrigerated overnight at 1° C. and tested at 2° and 8° C. Worms were tested as usual in shallow dishes of sea water at 15·5° C. The pieces of stomach were removed from the refrigerated sea water and rapidly presented to the heads of the commensals. Before each worm was solicited, the piece of stomach was briefly put back into the refrigerated sea water to chill its surface again.

Worm	2° C. stomach	8° C. stomach	15·5° C. stomach (control)
1	0	+	+
2	0	+	+
3	0	(+)	+
4	(+)	+	+
5	(+)	+	+
6	(+)	+	+

As can be seen, a weak response was elicited from half the worms at close to freezing temperature, while stomach at 8° C. seemed as effective as controls.

Experiment No. 7. How do high temperatures affect the ability of whole stomach to elicit a response? Pieces of stomach were placed in sea water at 44° and 64° C. for 10 min., then placed in sea water at the temperature of control stomach (15·5° C.) to cool them off, and presented, with results as follows:

Worm	15·5° C. stomach	44° C. stomach	64° C. stomach
1	+	+	0
2	+	0	0
3	+	(+)	0
4	+	+	0
5	+	+	0
6	+	0	0

The data indicate that raising the temperature to 44° C. either slightly alters the attractant or allows just about the quantity to be released which is at the threshold sensitivity of the worms. Temperatures higher than 64° C. either completely destroy or alter the attractant, eliminating all response.

It is apparent, then, from the above experiments that passage of time or reduction of temperature do not markedly alter the attraction of whole isolated host tissue, but that temperatures much higher than 45° C. cannot be used in efforts to extract the attractant factor.

Experiment No. 8. What effect does grinding up the tissue have? About twenty *Astropecten* arms were ground up in a Waring Blendor in 150 ml. of 'outside' sea water. This material was then centrifuged at 1500 r.p.m. for 15 min. Cotton-wool was soaked in the supernatant and presented. No response whatever occurred, while control whole arms elicited typical responses from all six commensals.

Experiment No. 9. Five *Astropecten* stomachs were ground up in 5 ml. of clean sea water and sand and passed through filter-paper. Presentation of cotton-wool soaked in this filtrate resulted in no response whatever. Control pieces of whole stomach elicited typical responses from all commensals.

Experiment No. 10. A large number of tube-feet were rapidly ground up in 5 ml. of clean sea water and sand. This material was centrifuged at 1500 r.p.m. for 15 min. and the supernatant presented soaked in cotton-wool. Again, there were no responses whatever.

Experiment No. 11. What if the ground material is not filtered? Five *Astropecten* stomachs were ground up in 8 ml. of clean sea water in sand and the preparation allowed to stand for a few minutes to settle out, before presentation:

Worm	Control stomach	Control cotton-wool	Cotton-wool soaked in preparation
1	+	0	(+)
2	+	0	0
3	+	0	(+)
4	+	0	0
5	+	0	(+)
6	+	0	0

Here a weak response is occasionally elicited, which may result from contact with minute bits of tissue remaining in the supernatant. Clearly, no very effective amount of attractant remains in the preparation.

The above experiments indicate that grinding obliterates the attraction of host tissue and that the attractant present is rapidly altered or destroyed. Efforts to protect the attractant by introducing a crystal of ascorbic acid in the preparation or by grinding, centrifuging and testing at low temperatures (1–9° C.) resulted in failure.

However, two experiments gave evidence that the attractant is rapidly oxidized.

Experiment No. 12. Five *Astropecten* stomachs were isolated and placed in a test-tube with 8 ml. of sea water and a small quantity of washed sand. Through this preparation nitrogen was bubbled for 3 hr. At the end of this time the stomachs were quickly ground up in the nitrogen atmosphere, the supernatant poured off and tested:

Worm	Control stomach	Control cotton-wool	Cotton-wool soaked in preparation
1	+	0	(+)
2	+	0	(+)
3	+	0	(+)
4	+	0	+
5	+	0	0
6	+	0	+

Here again, as a result of the small sample tested and the appearance of a number of delayed or weak responses, one cannot make a definite statement on the relative effectiveness of the material which had been prepared in nitrogen. Yet the five responses, two of which were typical, appear to indicate that cotton-wool soaked in material prepared in nitrogen was slightly more effective than cotton-wool soaked in material prepared in air (Exp. No. 11).

Experiment No. 13. Finally, cotton-wool soaked in stomach contents and then exposed to air in a moist chamber was found very quickly to lose its attraction. Bits of cotton-wool were placed in the stomachs of hosts for

90 min., quickly removed, placed in a moist chamber in air, and tested. After the passage of 5 min. in air bits elicited normal responses, but others which were tested after they had been in air for 15 min. had lost all their attraction.

GATTYANA CIRROSA (PALLAS) AND *LEPIDASTHENIA ARGUS* HODGSON

The association of these two handsome polynoids respectively with the terebellids *Amphitrite johnstoni* Malmgren and *A. edwardsi* Quatrefages is well known.

In a series of experiments, with material collected at the estuary of the Yealm and at Salcombe, the ability of the commensals to discern the presence of their host terebellids at a distance was tested with the Y-tube choice-apparatus used in the author's 1950 and 1951 experiments. In no tests was it possible to demonstrate that the commensals chose the host-water arm a statistically significant number of times, even when as many as seven or eight host terebellids were placed in the test aquarium, when 'outside' sea water was used and when the commensals were partially protected against the adverse effect of light stimuli by wrapping the Y-tube in red cellophane.

This result strictly accords with that obtained (Davenport & Hickok, 1951) with another terebellid-polynoid association, that of *Amphitrite* (*Neoamphitrite*) *robusta* (Johnson) and *Halosydna brevisetosa* Kinberg of Puget Sound.

Orton and Smith (1935), using *Gattyana* and *Lepidasthenia*, conducted brief experiments which they believed 'appear to indicate...that there is a tropic response on the part of the polynoid, causing it to enter an *Amphitrite* burrow whenever possible.' No data is presented, however, to support this hypothesis. In a personal communication on unpublished work R. Phillips Dales states that he has been able to demonstrate a positive response by *Gattyana* to water from the host, when using only the freshest material and taking the greatest precautions to prevent the disturbing effects of other stimuli (light, contact, etc.). It is clear, however, that while in these terebellid-polynoid associations the commensal's behaviour may be affected by a chemotaxis to the host, certainly not nearly as strong a response can be demonstrated as in the echinoderm-polynoid partnerships. The author has been unable to duplicate, when using *Amphitrite johnstoni* and *Gattyana*, his experiments demonstrating the response to host body wall or tentacle exhibited by *Halosydna brevisetosa*.

Observations made in the field and on material in glass U-tubes indicate that the activity of commensals is perhaps limited more by the powerful thigmotaxis they demonstrate to the wall of the tube and the body-wall of the host than by responses to attractants secreted by the host.

That a chemotaxis may be involved in enabling the young commensals to find their hosts cannot be discounted.

DISCUSSION

In the above experiments the importance of an unstable attractant in regulating the behaviour of the commensal partner in an association has become apparent for a second time. A marked difference exists, however, between the control of the *Evasterias-Arctonoë* partnership of Puget Sound and that of the *Astropecten-Acholoë* partnership of European waters. In both of them the commensal annelid is bound to the host asteroid by a powerful chemotactic response to an unstable substance or substances; but, whereas in the former the attractant will take effect at some distance from the host, in the latter a response can be elicited only by contact with the host. That the response of *Acholoë* may be purely a chemotaxis, in spite of contact being necessary, has been strongly suggested by the tests in which it was elicited by stomach juices soaked on cotton-wool.

Unfortunately in the 1950 Puget Sound experiments no tests were made similar to those in which *Acholoë* was found to respond to several starfish that appear never to be its natural host; however, in the Y-tube choice-apparatus used, *Arctonoë* was found not to respond to water from the star *Pisaster*, closely related to the host *Evasterias* and living in the same ecological niche. The lack of specificity of response in *Acholoë* is difficult to explain in view of the fact that it has been found only on *Astropecten* and *Luidia* in European waters.

It must be kept in mind, however, that under ordinary conditions during adulthood of the commensal, the chemotaxis serves merely to keep the commensal on the surface of the host. In a personal communication, G. Thorson, who has recently made some interesting observations on this partnership and to whom I am indebted for the excellent figure of the animals, says that when *Astropecten* are dying in an aquarium their *Acholoë* will leave them and may visit *Asterina*. This is quite in accord with our observations of the sensitivity of *Acholoë* to *Asterina*, on which the polynoid never occurs in nature. Under natural conditions an annelid during its adult lifetime would seldom be forced to seek a new host when its own is damaged or dying. Such an event is probably too rare to be considered a factor in the evolution of the powerful chemotactic response; but its occasional occurrence could conceivably initiate a new host habit, when the searching commensal finds itself able to 'colonize' a new host species.

Probably no answer will be found to the problems of the observable differences in the distances at which particular commensals respond, of the specificity of their responses, and of their specificity of habit, until the detailed ecology of the developmental stages of the respective commensals is known, including the manner in which the host is sought out and found.

During study of the effect of the host on the early stages of the commensal, Thorpe's (1939) olfactory conditioning factor must be kept in mind as a possible important element in the control of marine associations. In this work Thorpe demonstrated that in certain insects host selection in part depends upon the conditioning of the parasite's early stages to host substance. For such conditioning to be effective the early stages must obviously spend some time in intimate contact with host tissue. Work at present in progress indicates that the prototrochophore and trochophore stages of *Acholoë* are in the plankton for not less than 10 days. It is a little difficult to see how any conditioning of the early stages can occur in forms with as long a planktonic stage as *Acholoë*.

In considering the evolution of a specific commensal habit it is admittedly dangerous to generalize from an evaluation of the economics of the association based largely on guess-work. It should be possible to determine precisely, in a number of partnerships, just how the commensal is attracted and bound to the host and how the host is forced to tolerate it. Turning to the *consequences* of such an association, the remarks of Maurice Caullery (1952), referring to lichens, are quite as applicable here. He says that we will 'find that the question is really one of analyzing, by precise experiments, the relations' (of the partners) 'and of careful comparison of their behaviour in an isolated state and in association.' Such a careful comparison of the relations and requirements of the partners of specific associations of the type investigated by the author has to his knowledge never been made.

A possible course of evolution of intimate animal associations in general may be suggested. When one animal becomes associated with another to the advantage of the former, then the latter (the host) may have a number of possible fates. It may be able to evolve defences against the partner about as fast as the partner is able to evolve modifications to live at its expense, in which case the association may be considered one of commensalism or balanced parasitism. Or it may not be able, and the association may then be thought of as one of extreme parasitism, internal predatism or disease. Or, again, if *after the association has been unilaterally initiated*, some benefit is thereby given to the host, the host individuals which are best able to take advantage of the situation will perhaps be selected and the association will then tend to become truly mutualistic or symbiotic. However, it is difficult to take any other view than that at the initiation of the association only one of the two associated species is benefited.

With the polynoid-echinoderm partnerships investigated by the author, it appears most unlikely, since such a high percentage of host individuals live quite successfully without commensals, that some selective advantage has accrued to species which developed a substance whereby commensals were attracted. It is most difficult to imagine any way in which the echinoderm benefits from this association and therefore to label the partnership mutualistic. Should not then—the question is often put—these echinoderm-annelid associations be considered parasitic? If commensalism is defined classically as simply 'feeding at the same table', and if it can be demonstrated that no damage is done to the starfish by a commensal that occasionally removes food from its stomach, the answer is 'no'. Obviously, however, these associations are very close to parasitism and probably are excellent examples of the manner in which truly parasitic associations develop.

In the author's opinion, from the point of view of the evolution of the association, the echinoderm has been passive. It has, in the course of the annelid's adaptation to commensal life, been unable to eliminate the commensal.

On the other hand, an organism that has become physiologically adapted both to finding, by evolving a positive response to a particular chemical secreted by a host, and to successfully 'colonizing' this host, has doubtless been given an advantage of selective importance. The finding itself and the successful 'colonization' must be investigated without undue regard to the economics of the association and with careful consideration of the manner of evolution of sensitivities to specific substances and, undoubtedly, of certain immunities. The author has observed *Acholoë* fully two-thirds within the stomach of the host, and Thorson states that they will keep this position for 10 min. or more. The annelids must be quite immune to host digestive enzymes. It may be that immunological studies will cast light on the apparent anomaly of a commensal that responds positively to a number of starfish (perhaps biochemically related) but is found in nature on only one or two of these.

That various intimate associations among terrestrial organisms (parasitic protozoa, trematodes, insects, etc.) are controlled by chemical sensitivities and immune reactions is well known; so far little attention has been paid to the role of these phenomena in marine associations.

SUMMARY

The commensal polynoid *Acholoë astericola* (Delle Chiaje) exhibits a powerful positive response on contact with its host, *Astropecten irregularis* (Pennant).

This response is only relatively specific, *Acholoë* demonstrating it to a number of starfish that do not serve as host in Plymouth waters.

All living host tissue has some attraction for the commensal; and the attractant is apparently released in quantity in the stomach of the host.

Passage of time (apparently as long as tissue remains alive) and chilling do not markedly alter the attractiveness of isolated host tissue. Temperatures above 45° C. alter or abolish its attraction.

The extreme instability of the attractant has so far made it impossible to demonstrate its presence in extracts.

Evidence is presented that the attractant is rapidly oxidized.

Experiments with the scale-worms *Gattyana cirrosa* (Pallas) and *Lepidasthenia argus* Hodgson, commensal respectively with *Amphitrite johnstoni* Malmgren and *A. edwardsi* Quatrefages, indicate that a chemotaxis to the host may be of relatively minor importance in governing the behaviour of these commensals.

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SOME EXPERIMENTS ON THE FUNCTION OF THE PERICARDIAL ORGANS IN CRUSTACEA

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(Text-figs. 1–11)

The experiments reported here were designed to test the assumption that the nervous organs found in the pericardial cavity of the Crustacea, and termed 'pericardial organs'[1] (Alexandrowicz, 1953*a*, *b*), have a secretory function. Their situation suggested that the substances liberated by them into the blood of the pericardial cavity probably act on the heart. Accordingly, the aim of this series of experiments was to determine what might be the effect of these postulated hormones on the crustacean heart.

Method

The method employed consisted in preparing an extract of the pericardial organs and testing its action by adding it to the fluid perfusing the isolated heart.

The observations were chiefly made with the two species of large crabs easily available at Plymouth, *Cancer pagurus* and *Maia squinado*. The lobster (*Homarus vulgaris*) proved to be less suitable experimentally. Some experiments were performed at Naples[2] with a stomatopod, *Squilla mantis*. There were perfused in all 15 hearts of *Cancer*, 26 of *Maia*, 4 of *Homarus* and 10 of *Squilla*.

As pointed out in the paper quoted above, the pericardial organs can be found most easily in the pericardial cavity of crabs, where they lie at the openings of the branchio-cardiac veins. With *Maia* there is a chance of removing all the elements of the organs of one side hanging together, i.e. the anterior and posterior bars with their prolongations and the longitudinal trunks. In *Cancer* the organs must be taken out in two portions separately, i.e. the anterior and posterior bars each with the adjoining parts. This procedure is also often more convenient in *Maia* since it is easier, and since in the preparation of extracts the organ need not be in one piece.

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[1] R. I. Smith in a figure illustrating the course of the cardiac nerves in *Cancer irroratus* (*Biol. Bull. Woods Hole*, 1947, Vol. 93, p. 77) has shown nerves designated as 'lateral pericardial plexus', which are certainly the elements of the pericardial organs. This 'lateral pericardial plexus' was also observed in *Pugettia producta* by J. P. Heath (*Journ. Morph.*, 1941, Vol. 69, p. 481). The term 'pericardial organs', proposed by one of the present writers (J. S. A.) in his previous publication, and adopted without knowledge of the designation previously given, seems more appropriate in view of the unusual structure and function of these elements: this term will also embrace the analogous elements in stomatopods first described as parts of the 'system of dorsal trunks'.

[2] We wish to express our gratitude to Prof. R. Dohrn for the facilities offered to us. One of us (D. B. C.) is indebted to the Browne Fund of the Royal Society for financial assistance, which made his visit to Naples possible.

The isolated organs were first rinsed in the saline used for perfusion and then triturated with sand in a little of the same solution. The suspension was filtered and the residue washed with several more portions of fluid totalling 10 ml. The filtrate was used, in most of the experiments, as the stock solution from which certain amounts were taken and added to the perfusion fluid. The stock solution was prepared fresh before each series of tests.

In *Homarus* only a part of the pericardial organs is arranged in discrete trunks: the other part is spread as plexuses over the wall of the pericardium. Moreover, even the trunks themselves, which are much shorter than in crabs, cannot be easily distinguished from other tissues. As the dissection is difficult and its result uncertain only a few trials were made.

In *Squilla mantis* the pericardial organs are present in the 1st–5th abdominal segments and are of two kinds. Those of one kind, short trunks flattened to lamellae spanning the cleft between the dorsal muscles, can be easily found. To expose them, parts of the terga have to be removed, care being taken not to tear away the tissues in the mid-line. The lamellae are recognizable by their shape and position, sometimes also by their slightly greyish colour differing from the yellowish hue of the muscles. The elements of second kind, spreading over the pericardial wall, are in practice scarcely distinguishable in unstained preparations.

The saline used for perfusion in all four species was prepared according to the formula of Welsh (1939*a*) (modified Pantin's solution). The perfusion of the heart of the decapod crustaceans was performed according to the method used by Welsh (1939*a*) with some minor modifications of the apparatus and procedure.

The routine was as follows.

(i) The legs were cut off and the dorsal part of the carapace and of the pericardium removed.

(ii) The pericardial organs were exposed by cutting the lateral heart ligaments. The organs were excised and the extracts prepared.

(iii) The heart, with parts of the arteries, was cut out and put into a flat dish with perfusion fluid to rinse. Sea water may also be used for rinsing the heart, as it seems not to affect its later behaviour.

(iv) A cannula already fixed into the rubber stopper was introduced into the heart through the sternal artery. A cannula with a spherical enlargement of the tip may meet resistance at the arterial valve, and only when it can be pushed freely farther into the heart cavity is there a certainty that the valve has been passed.

(v) A ligature was tied round the sternal artery to fix the cannula in place, and a second one round the anterior aorta.

(vi) The heart was put into a short glass tube with a capacity of 80 ml., the stopper fixed in place, the tube filled with perfusion fluid, and the thread of the anterior ligature attached to the writing lever.

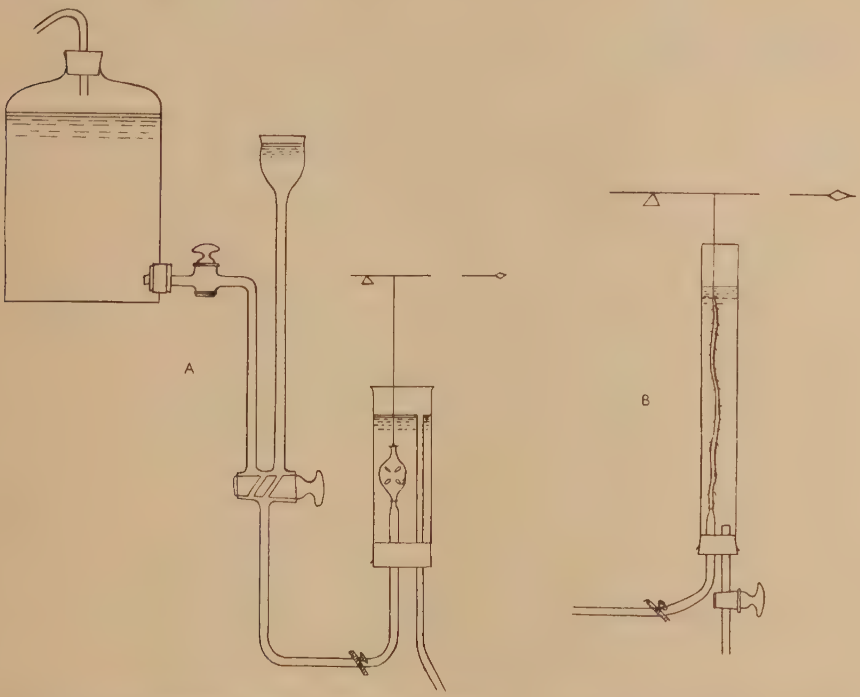

Fig. 1. A, the apparatus used for perfusing the hearts of *Cancer*, *Maia* and *Homarus*. The Mariotte bottle was only used for prolonged washings. Before, during and for a short time after each test perfusion was made from the thistle funnel, with constant addition of fluid. The rate of perfusion was controlled by means of the screw clamp. B, the modification of the heart chamber used for perfusing the heart of *Squilla*, drawn to a larger scale than A.

(vii) Perfusion was begun first from the Mariotte bottle (Fig. 1 A), but, as differences in the level of the perfusion fluid may cause some changes in the heart rhythm, the two-way stopcock was turned before each test and the perfusion was made for some time from the thistle funnel into which later was poured the saline with the substance to be tested. In this way the same conditions of perfusion prevailed before, during and after each test. The Mariotte bottle was only used for prolonged periods of unattended perfusion and for washing out test solutions.

The volume of each test solution, i.e. saline with the substance to be tested added, was as a rule the same, viz. 20 ml.

To expose the heart of *Squilla mantis* the dorsal parts of the terga and of the extensor muscles must be removed as far as is necessary to give free access to the long heart tube. The latter can then be taken out, preferably from the hind end forwards, by successively cutting the arteries and the connexions with the underlying organs. The heart can be completely isolated, but it is safer to leave it with the gonad attached. So far as could be observed this does not influence its reactions. After the tip of the heart has been cut off close to the posterior aorta, the cannula can be introduced and secured by a ligature. A thread put round the anterior end of the heart serves to attach it to the writing lever.

The heart of *Squilla* is pierced by thirteen pairs of ostial orifices and sends off, apart from anterior and posterior aortae, fifteen pairs of arteries. The conditions for perfusing such a heart are obviously very unfavourable. Moreover, the ganglionic trunk is situated not on the inside but on the outside of the dorsal heart wall, and therefore it is not easily accessible to the perfusing fluid from the inside. Therefore, in order not to dilute too much the substances to be tested in the fluid bathing the heart, the tube used for perfusion was narrower than in experiments with other crustaceans and contained only 20 ml. By means of the tap (see Fig. 1 B) the fluid could be kept at a level just covering the top of the heart, and through the same tap the contents of the tube could be completely evacuated. When refilled the tube contained only traces of the solution previously tested. During change of fluid the heart action is temporarily disturbed, but afterwards resumes normally.

With few exceptions, for which no good reason could be found, the heart, removed from the animal body, and often quite motionless during the dissection, started beating as soon as it was immersed and perfusion began. At each contraction the heart tube becomes twisted so that its top moves clockwise round the vertical axis. This movement is obviously due to the spiral course of the muscle fibres; the vertical component of the contractions, however, has sufficient amplitude to give quite satisfactory records.

ACTION OF EXTRACTS OF THE PERICARDIAL ORGANS AND OF DRUGS

Cancer pagurus

Action of Pericardial Organ Extracts

The addition of extract of its own pericardial organs to the perfusion fluid has a very distinct effect on the heart beat of *Cancer*, increasing the frequency and amplitude of the contractions. This effect proved to be dependent on the activity of the extracts and on the sensitivity of the heart, both factors being subject to individual variations. The record in Fig. 2A was obtained with a concentration of 0·4 ml. of the stock solution diluted to 20 ml. On occasion a distinct reaction was noted with 0·1 ml. of the stock solution of the extract, i.e. using 1/100 of the active substance of the organs of one animal, diluted in 20 ml. of saline. In other experiments with other hearts and extracts greater quantities, up to 4 ml. of the stock solution, were needed to produce a similar reaction.

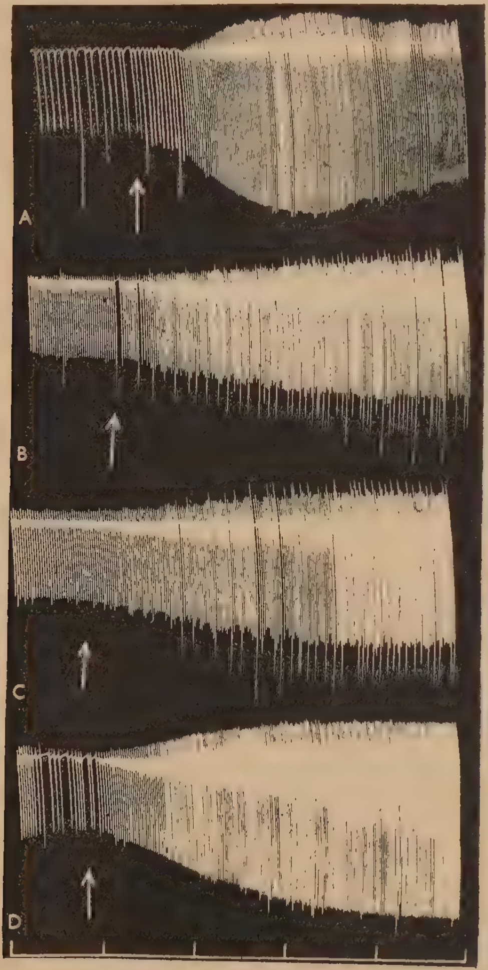

Fig. 2. The successive action on the same heart of *Cancer* of (A) pericardial organ extract of *Cancer*, (B) adrenaline ($1:10^6$), (C) noradrenaline ($1:10^6$) and (D) the same extract as in A. The records read from left to right; the arrows indicate approximately the time at which the reagents reached the heart; time scale in minutes.

The effect of the extract in exciting the heart beat is not long-lasting and after all the fluid containing the extract has passed through the heart and been replaced by ordinary saline the heart gradually returns to its previous rhythm.

Extracts of pericardial organs of *Maia squinado* (Fig. 3) and of *Portunus puber* produced similar reactions in the *Cancer* heart. To obtain effects equal to the above with the *Maia* extracts a higher average dose was required, indicating that in the organs of this animal there is less accelerating principle. With *Portunus* comparisons are uncertain, not only because of its much smaller size, but also because the dissection of its pericardial organs is difficult and probably loses much of their parts.

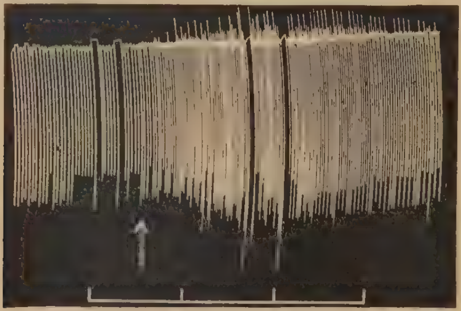

Fig. 3. The effect of pericardial organ extract of *Maia* on the heart of *Cancer*. The record reads from left to right; the arrow indicates approximately the time at which the extract reached the heart; time scale in minutes.

Action of Adrenaline, Noradrenaline and Tyramine

The action of adrenaline and acetylcholine on crustacean hearts is known through investigations of several writers (Hogben & Hobson, 1924; Bain, 1929; Welsh, 1939*a*, *b*; 1942; Davenport, 1941; Ghiretti, 1947), and there was no need to control the established facts. We tried these drugs, however, in order to be able to compare their effects with those produced by the extracts of the pericardial organs on the same hearts and under the same conditions.

Of the drugs tested, adrenaline (Parke, Davis and Co.) and noradrenaline (Levophed, of Bayer), at concentrations of $1:10^6$ or $1:10^7$, showed effects most resembling those of the extracts. These two drugs and the extracts were so much alike in their effect—an acceleration of the beat and a gradual increase in amplitude, sometimes to remarkable maxima, followed by an equally gradual return to normal—that often no differences could be noticed. However, some hearts appeared to react somewhat differently in response to the extracts from their reaction to the drugs. Fig. 2 shows records obtained with the same heart treated (A) with the extract, then (B) with adrenaline, (C) with noradrenaline and (D) again with the same extract, the last trial to eliminate the possibility of the previous treatment having influenced the response of the heart to that following. It will be noticed that the extract, apart from the increase in frequency and amplitude, has also a steadying effect on the beat, indicated by more even contractions.

The action of acetylcholine is different (Fig. 4). It accelerates the heart rhythm and may also increase slightly its amplitude; at higher concentrations, however, the amplitude not only does not become greater, but markedly decreases, becoming even smaller than during the previous 'normal' beat till the heart stops in diastole or nearly in this condition. On the latter point our observations on *Cancer* and *Maia* differ from those of Welsh (1939*a*, *b*), Davenport (1941) and Ghiretti (1947), who recorded stoppage not in diastole but in systole. In conversation with Dr J. H. Welsh we have learned that in his experiments he used a counterbalanced writing lever, whereas in the experiments reported in this paper we used a spring-loaded lever, so that the heart was always pulling against the tension of a spring. This may explain the different conditions of stoppage after acetylcholine in the two sets of experiments.

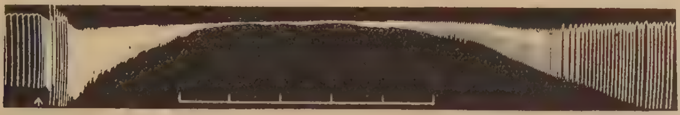

Fig. 4. The effect of acetylcholine (1:10^3) on the heart of *Cancer*. As in all the kymograph records reproduced here, contractions are downwards so that the stoppage of the heart has taken place in diastole. The record reads from left to right; the arrow indicates approximately the time at which the drug reached the heart; time scale in minutes.

The experiments with tyramine-hydrochloride (L. Light and Co.) gave uncertain results. In some a slowing action of this drug at concentrations of 1:10^7, 1:10^6 or 1:10^5 was recorded; in others it was ineffective at concentrations of 1:10^6 and slightly accelerating at 1:10^5. Since the effects obtained do not resemble those of the extracts, this substance is not likely to prove the active principle of the organs in question.

The assumption that the active principle may be adrenaline-like was corroborated by the fact that pericardial-organ extract from *Cancer*, after addition of sodium hydroxide, gave a distinct fluorescence reaction of the same green colour as adrenaline. The intensity of the reaction shown by the extracts of organs of a single specimen prepared with 10 ml. saline corresponded approximately to a 1:10^6 solution of adrenaline. On the assumption that this principle would be released by the organs into the pericardial cavity, we tested blood taken from this cavity and found it to give a fluorescence of the same kind. This reaction, however, was not shown by blood of the same animal taken from the leg arteries. The extracts of the organs and the blood of *Maia* either did not give the fluorescence reaction at all or at the most very slightly. The extracts from the organs of *Portunus* gave a distinct positive fluorescence reaction.

It follows from these experiments that the pericardial organs in decapod crustaceans release some adrenaline-like principle which has an excitatory effect on the heart beat. Experiments with another crab, *Maia squinado*, show, however, that this is not a complete statement of the action of these organs.

Maia squinado

Action of Pericardial Organ Extracts

The action of the pericardial-organ extract on the heart of *Maia* is strikingly different from that in *Cancer* for it decreases the frequency of the beat. The amplitude becomes only a little greater and never reaches such a height as in *Cancer*, and the higher concentrations do not bring about a further increase.

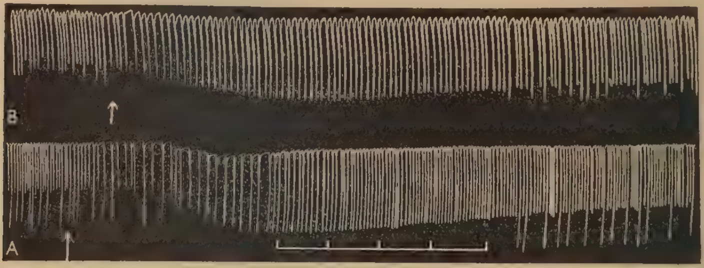

Fig. 5. The effect on the heart of *Maia* of its own pericardial organ extract. A shows the fourfold action—decrease in frequency and increase in amplitude of the beat, increase in tonus and the greater regularity of the beat, with a return to its previous condition as the extract is washed out of the heart; B shows more clearly the steadying effect of the extract, and in this heart there was no increase in tonus. The records read from left to right; the arrows indicate approximately the time at which the extracts reached the hearts; time scale in minutes.

Other effects, not always noticeable, are the steadying of the beat and an increase in tonus. The fourfold action of the extracts may be seen in Fig. 5A. The steadying effect is also well illustrated by Fig. 5B showing how the uneven beat of the heart became more regular after treatment with the extract and, after cessation of the action of the extract, returned to its previous rhythm with the same irregularities.

The heart in *Maia* proved to be less sensitive than in *Cancer*, and for recording the action of the extracts 2–5 ml. of the stock solution were made up to 20 ml. of the perfusion fluid.

The result of the perfusion of *Maia* heart with the extract of *Cancer* is shown in Fig. 6. The heart responded by an increase in amplitude and by a slight decrease in frequency of beat. This shows, therefore, that the hearts of the two species react differently from each other to their own respective pericardial extracts, and these differences are maintained when the hearts are subjected to treatment with extracts from the opposite species.

Action of Drugs

The heart of *Maia* responds to adrenaline by an increase in frequency and amplitude of beat. It will, however, be noted that *Maia* heart is less sensitive than *Cancer* heart to this principle in reacting with less vigorous contractions; the effect shown in Fig. 7 is rather exceptional and different sensitivities of the hearts could often be observed. Thus the reaction in one heart was just distinct with a concentration of 1 : 10^8, whereas in another no effect was noted with this concentration. The action of noradrenaline is similar to that of adrenaline, but sometimes somewhat stronger contractions were observed after treatment with noradrenaline than with adrenaline of the same concentration.

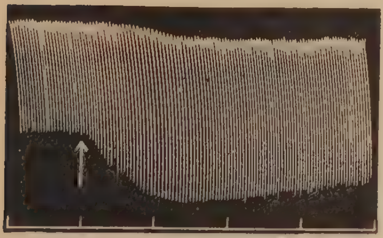

Fig. 6.

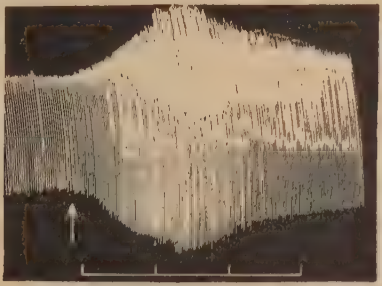

Fig. 7.

The records read from left to right; the arrow indicates approximately the time at which the active principle reached the heart; time scale in minutes.

Fig. 6. The effect of pericardial organ extract of *Cancer* on the heart of *Maia*.
Fig. 7. The effect of adrenaline (1 : 10^6) on the same heart of *Maia*.

The action of acetylcholine does not show marked differences from that on the heart of *Cancer*.

Tyramine in higher concentrations, such as 1 : 10^5, first slows the beat and decreases the amplitude and then accelerates the beat and increases the amplitude. In lower concentrations, such as 1 : 10^7, a slowing of the beat was recorded. The regular heart rhythm seems to be affected by this drug and occasionally the heart stops for a while. Tyramine thus seems unable to play the main role in the action of the pericardial organs.

Homarus

Because of the arrangement of the pericardial organs in *Homarus* only a small part of them could be cut out, and the extracts contained, at best, only a small fraction of the active substance presumably present in the whole of the organs. Applied to the heart of *Homarus*, which was perfused in the same way as those of crabs, the extracts increased the frequency and amplitude of beat (Fig. 8 A); both effects however being less pronounced than in *Cancer*. Extracts from *Maia* and from *Cancer* both have an excitatory effect and no marked difference in the response to either was noticed (Fig. 8 B, C). Even when a certain *Homarus* heart showed a peculiar reaction by first increasing the frequency and then slowing it abruptly the records were practically identical with *Maia* and with *Cancer* extracts. In general the heart of the lobster

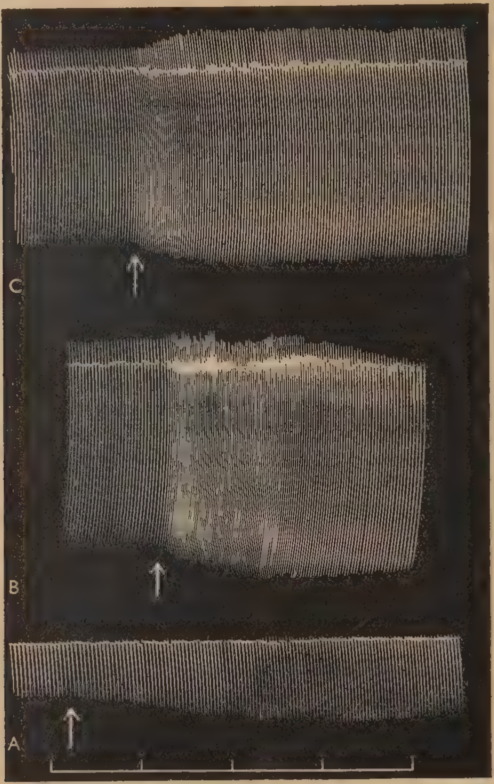

Fig. 8. The effect on the heart of *Homarus* of (A) pericardial organ extract of *Homarus*, (B) pericardial organ extract of *Cancer*, and (C) pericardial organ extract of *Maia*. Records read from left to right; the arrows indicate approximately the time at which the extracts reached the heart; time scale in minutes.

appeared to be less sensitive to the extracts and to adrenaline and noradrenaline as well. The threshold concentration of both these compounds was as high as $1:10^5$.

Squilla mantis

Obviously, owing to its anatomical features, only a part of the inside of the heart of *Squilla* can come into direct contact with the undiluted stream of perfusion fluid, and therefore, if some substances are added to this fluid, they can act on remaining parts of the heart elements only after being diluted by the fluid already filling the container. Therefore even negative results would not be decisive. Fortunately, it was found that extract made from the pericardial organs of a single specimen (five lamellae) was sufficient to cause a distinct increase in frequency and amplitude of beat (Fig. 9A). Similar effects were brought about by adrenaline and noradrenaline (Fig. 9B). The concentration of these substances in the perfusion fluid was $1:10^6$, but, as just pointed out, the concentration at the moment of their action on the receptive elements of the heart was uncertain.

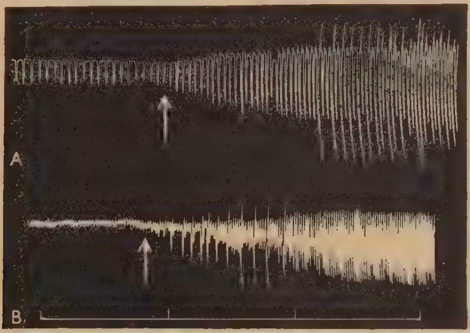

Fig. 9. The effect on the heart of *Squilla* of (A) pericardial organ extract of *Squilla*, and (B) adrenaline ($1:10^6$). The records read from left to right; the arrows indicate approximately the time at which the reagents reached the heart; time scale in minutes.

The action of the extracts from crabs on *Squilla* heart, and vice versa, could not be determined, since all these animals were not available at one and the same place, and we had not yet worked out a reliable method of preserving the active extracts for future use.

The fluorescence test with the pericardial organs of *Squilla* gave uncertain results. At this stage of the work it could not be determined whether the active principle in *Squilla* is different from that in *Cancer* or too small in amount to give the reaction.

TESTS ON RELEASE OF SECRETION INTO THE BLOOD STREAM

In order to test the supposition that the pericardial organs release the active substance into the blood the following experiments on crabs were performed.

Perfusion of the heart with blood. Blood was taken, one sample from the leg arteries and another from the pericardial cavity of the same animal. Each sample was filtered and, after dilution with an equal amount of saline, tested on the heart. The result of this experiment in *Cancer* (Fig. 10) shows that the blood from the pericardial cavity has a similar stimulating effect to the extracts of the pericardial organs, whereas that from the leg arteries slows the heart beat and decreases its amplitude.[1]

Perfusion of the pericardial organs in situ. An animal was bled through the leg arteries and then from the pericardial cavity through a small hole bored into the carapace above the heart. After the animal had been fixed by means of a clamp with the ventral side upwards, the pericardium was perfused through three tubes introduced into the branchio-cardiac veins. The fluid flowing out through the hole in the carapace was collected and tested on the heart of a second animal perfused in the ordinary way. In experiments made with *Cancer* this fluid showed a distinct stimulating effect (Fig. 11) and gave a positive fluorescence reaction.

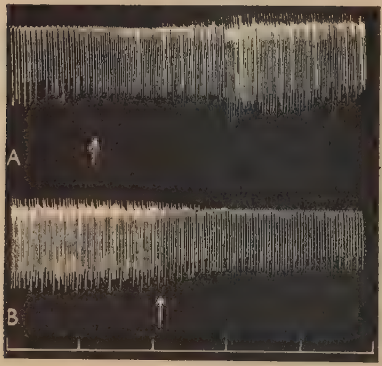

Fig. 10.

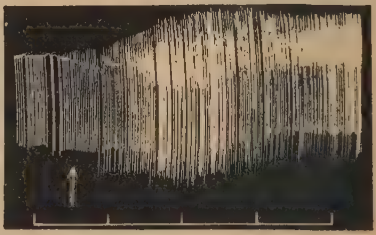

Fig. 11.

The records read from left to right; the arrow indicates approximately the time at which the active material reached the heart; time scale in minutes.

Fig. 10. The effect of blood on the heart of *Cancer*. The blood was filtered after clotting and the filtrate diluted with an equal volume of saline. A, blood taken from the pericardial cavity stimulates the heart; B, blood taken from the leg arteries depresses it.

Fig. 11. The effect on the heart of *Cancer*, of saline which had been perfused through the pericardial cavity *in situ*, passing over the pericardial organs.

With *Maia* the results were uncertain. As the active substance of the pericardial organs and the blood both slow the beat, the differences in their action

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[1] Blood of crustaceans taken out from the animal body ceases to be a proper medium for the tissues: it affects the heart action, decreasing the amplitude of the beat, and often causing an irregular rhythm and sometimes even stoppage of the heart. This is obviously due to the quickly occurring changes in the composition of the blood.

(an increasing of amplitude by the former and decreasing by the blood) are hardly distinguishable. Moreover, the exactness of the experiments with perfusion *in situ* is somewhat impaired by the fact that, even after a rinsing of the pericardial cavity prior to collecting the fluid for the tests, some of the blood remains there and passes into the perfusion fluid, revealing its presence by an opalescence. The presence of blood may influence the reaction of the heart on test, but the preliminary washing of the pericardial cavity cannot be prolonged lest the pericardial organs should be exhausted.

Possible Errors

As noted by other workers in this field, allowance must be made for the hearts taken out of various specimens behaving in quite different ways. Some hearts start to contract strongly and in fairly regular rhythm immediately on being immersed in the saline and after the stream of perfusion fluid passes the cavity of the heart; others need some time to adjust themselves to these conditions. Several did not contract at all for a long time, but did so after addition to the perfusion fluid of a small amount of the pericardial extracts or of adrenaline, and then continued to beat regularly for several hours. The degree of stretching of the heart and the rate of perfusion must be found empirically. We tried at first, in order to compare the action of various substances under conditions as similar as possible, to maintain a constant rate of perfusion, viz. 20 ml. of the solution through the heart in 3 min. This was approximately the rate of flow in most of the experiments, but various hearts show individual predilections, and even the same heart, after beating for some time at a certain speed of perfusion, may require later a faster one. Usually the hearts respond to the accelerated perfusion with stronger contractions. However, even this rule is not without exception: sometimes the heart worked definitely worse at the flow-rate usually used and needed one much slower.

This different behaviour may be partly due to blood clots forming in the heart and thus creating different conditions for passage of the perfusion fluid. It may be, too, that individual variations in the situation of the ganglionic trunk of the heart play some role. For instance, since this trunk lies on the inside of the dorsal heart wall and is partly covered by the muscle bundles, it is possible that, because of variations both in the thickness of these bundles and in the position of the ganglion cells, this pacemaker may be more or less accessible to the action of the mediators. The thickness of the muscle bundles is perhaps responsible for less satisfactory results with the hearts of the largest specimens of *Maia*. We preferred, therefore, to use medium-sized animals (carapace measuring about 13 × 17 cm.), rather than large ones.

The results of the experiments depend not only on the variable sensitivity of the hearts but also on an even more unstable factor, the activity of extracts, which appear to vary greatly, presumably in relation to the condition of the animals.

All the experiments recorded above were made during the summer months. When we tried to continue this work in January, however, the results obtained were unexpectedly confusing. A concentration of *Maia* organ extracts several times higher than that always found previously to be effective did not produce any reaction and even in one specimen slightly accelerated the beat.

It was then found that the hearts responded to treatment with adrenaline and noradrenaline in a way never previously observed by us and, moreover, in successive experiments conducted under identical conditions the results obtained were very different. Thus one heart did not react at all to a concentration of 1:5000; another reacted to a concentration of 1:10,000 by a slight acceleration without an accompanying increase in amplitude; again another, treated with a concentration of 1:1,000,000, responded by a spectacular increase in tonus, acceleration, and a notable decrease in amplitude. This was the same heart which accelerated in response to a highly concentrated pericardial organ extract. The reason for this abnormal behaviour of the hearts was possibly the low temperature at which the animals were kept in the outdoor storage tanks.

Although the experimental evidence speaks in favour of a hormonal nature of the substance released by the pericardial organs it can be objected that the active substances might be compounds of the tissues constituting the organs or of blood which pass into the extracts. It is known from experiments of Welsh (1939*a*) that nervous tissue from both the central nervous system and from peripheral nerves has an effect on the heart, and, as this writer concluded, 'the active principle in question is, with little doubt, acetylcholine'. We tested the effects of an extract of leg nerves of *Maia* and found that these were the same as in Welsh's experiments, and certainly different from those produced by the extract of the pericardial organs. Pieces of pericardial wall and blood clots even much larger than those which often adhere to the pericardial organs do not produce any effect.

Since on dissection of the pericardial organs in *Homarus* the removal of a certain amount of connective tissue with the organs was unavoidable, extracts from pieces of this tissue were made and tested but did not produce any apparent effect.

Discussion

The recorded results favour the assumption that the pericardial organs of the Crustacea contain some specific substances influencing the heart action. It must be emphasized that very small amounts of these substances are able to produce distinct effects, as may be inferred from the following estimation. The weight of the pericardial organs in a large specimen of *Maia* (carapace 17 × 20 cm., weight 1560 g.) was found to be 5 mg. and in *Cancer* (carapace 21 × 18 cm., weight 1810 g.)—8 mg. (Both organs were rinsed in saline and some of this fluid adhered to them during the weighing operation.) As stated before, 1/100 of the active substance of these organs diluted in 20 ml. was sufficient to show its action on the *Cancer* heart. Assuming that this substance made up as much as 5% of the total wet weight of the organs, which is certainly well on the high side, it is evidently efficient at a concentration of 1 : 5,000,000 or less.

In *Squilla* the weight of pericardial organs was not measured directly but from the dimensions of a lamella (about $1000 \times 300 \times 50\mu$) the weight of five of them can be supposed not to exceed 0·1 mg.; then the concentration of the active substance in 10 ml. of the perfusion fluid assuming the same 5% of total wet weight would be 1 : 2,000,000. As has been said above, this concentration was lowered by the fluid bathing the heart. Clearly then, in the investigations aiming at identifying the active principle, only such compounds as are capable of giving similar effects at very low concentrations ought to be taken into account.

There is one feature in the behaviour of the hearts in the two species of the Brachyura which requires particular consideration. Were the experiments performed with the heart of *Cancer* only, the results could be summed up in the statement that the pericardial organs in crabs (*Cancer*, *Maia*, *Portunus*) release a substance increasing the frequency and amplitude of the heart beat. Furthermore, the resemblance of the action of this substance to that of adrenaline and noradrenaline and the presence of a positive fluorescence reaction might lead to a conclusion that the function of the pericardial organs consists in liberating an adrenaline-like principle into the blood. The much weaker fluorescence reaction or its absence in the extracts from *Maia* would then show that the amount of this principle varies in different species of the Brachyura. From the experiments with the perfusion of the hearts in *Homarus* and *Squilla* the same or a similar principle would be admitted in the Macrura and the Stomatopoda.

The reactions of the *Maia* heart are similar in that the amplitude of the beat is increased and a stabilizing effect can sometimes be observed in both species of crabs. There is, however, a remarkable discrepancy in the chronotropic action, for the extracts from both *Cancer* and *Maia* accelerate the heart of *Cancer* and slow that of *Maia*. As the heart of *Maia* is accelerated by adrenaline it must be assumed that either (i) the substance in the extracts which stimulates the heart of *Cancer* is not adrenaline-like, but an unknown principle of such properties that it excites the heart in one species and inhibits it in the other, or (ii) the same stimulating principle is present in both extracts but its effect on *Maia* heart is inhibited by the presence of some other factor to which this heart is more sensitive. If the second alternative is regarded more probable, two possibilities have to be considered: this second factor could be either some non-specific substance of the tissues of the pericardial organs extracted together with the stimulating principle, or it is another hormone which, in the living animal, is also released into the blood of the pericardial cavity. The negative results of the experiments with the extracts of various tissues do not favour the first alternative. On the other hand, the inhibiting effects on *Maia* heart of small amounts of this substance seem to point to a hormonal nature. If this is so we reach the conclusion that the pericardial organs in the Crustacea liberate two hormones, one stimulating and the other inhibiting the heart rhythm. To the latter can perhaps be ascribed the stabilizing effect recorded sometimes in *Maia* and *Cancer*. The different reactions exhibited by the hearts of the two species of Brachyura could be explained in this way: that the heart of *Cancer* is more sensitive to the action of the accelerator and that of *Maia* to the inhibitor; and, when treated by the artificial mixture of the two, each heart responds according to its particular sensitivity.

In the other crustaceans investigated (*Homarus* and *Squilla*) the pericardial organs can be assumed to release a stimulating hormone, but the evidence so far available does not permit us to draw any conclusion as to the presence or absence of the inhibitory hormone.

Although the idea that the pericardial organs may have a neurosecretory function was based on the histological evidence alone, the latter did not help much in the solution of the problem whether these organs produce two different substances. The fine neuropile-like terminations of the nerve fibres, by which presumably the substances are given off into the blood, do not show any differences. It is, however, worth noting that in *Squilla* the nerves forming the pericardial organs are without doubt made up of two different sets of fibres and, as has been suggested in the description of these organs (Alexandrowicz, 1953*a*), their structure in the Decapoda may be the same, although here the diversity of origin of the nerve elements is not so easily discernible as in the Stomatopoda. If, therefore, it were proved that the pericardial organs in fact produced two hormones, the existence of two kinds of nerve fibres offers a possible explanation of their separate elaboration.

It appears from this work that in future physiological investigations of the crustacean heart the role of the pericardial organs must be taken into account. The experiments hitherto made on stimulation of the regulator cardiac nerves (for bibliography see Krijgsman, 1952) were as a rule performed after the pericardium had been opened and the possibility of noting the action of the pericardial organs thus eliminated. Only Parrot (1941) set up experiments in such a way that these organs could have produced their effect. This writer stimulated the inhibitory cardiac nerves in *Maia* in which the circulation was maintained, and took samples of the blood for testing on the intestine of the same animal. He found that such blood had a stimulating effect on the intestine and believed this to be caused by a substance released by the inhibitory nerves. This might have been so, but it seems more likely that in his experiments not only the inhibitory cardiac nerves but also the nerves of the pericardial organs were stimulated to release their hormones into the blood.

Possibly also in the experiments of Wiersma & Novitski (1942), in which the perfusion fluid was poured into the pericardial cavity, the pericardial organs had a chance to come into play; and conceivably their hormone was responsible for after-effects of inhibition supposed to be caused, as they wrote, by a 'liberation of a substance which can exert its influence at a later time'.

There seems to be no other instance when the action of the pericardial organs could be suspected of having affected the results of experiments (see the survey by Fredericq, 1947).

Summary

Experiments with isolated hearts of various Crustacea (*Cancer pagurus*, *Maia squinado*, *Homarus vulgaris*, *Squilla mantis*) were performed in order to test the postulated secretory function of the pericardial organs (nerve trunks of unusual structure lying in the pericardial cavity). Tests were made with extracts of these elements added to the fluid perfusing isolated hearts.

In *Cancer*, *Homarus* and *Squilla* the extracts caused a distinct increase in amplitude and frequency of the heart beat, and their effect proved to be very similar to that produced by adrenaline and noradrenaline tested on the same hearts. In *Maia* the extracts produced an increase in amplitude and decrease in frequency of the heart beat.

Extracts of the pericardial organs of *Cancer pagurus* gave the fluorescence reaction characteristic of adrenaline and related compounds. Blood taken from the pericardial cavity gave the same reaction, but that taken from the leg arteries did not.

It is assumed that the function of the pericardial organs in the Crustacea consists in liberating, through fine neuropile-like terminations of the nerve fibres, some hormone passing with the blood into the heart and producing on it a stimulating effect. There is certain evidence that the pericardial organs might release a second hormone having an inhibitory and perhaps also a stabilizing effect on the heart rhythm.

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A NEW DREDGE FOR COLLECTING BURROWING ANIMALS

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(Text-figs. 1–2)

At Plymouth the most successful dredgings are those from soft mud or shell gravel, grounds which offer little resistance to a heavy dredge of the normal pattern. From a sandy bottom reasonable samples cannot be collected because the ordinary rectangular dredge will not dig in more than, at the most, two inches. This fact, although it had long been inferred from the poor catches, has recently been proved by direct underwater observations.

A dredge of new design has been developed which will dig into the bottom, whether sand or mud, very rapidly and continue to dig the harder it is pulled. The name 'anchor-dredge' is proposed for this instrument. The basis of the design is a sharpened steel plate which is dragged at an acute angle to the sea-bed; this reduces the tendency for the sand to be broken up, as it is with most dredges, which are therefore left with nothing resistant to bite into. Once the anchor-dredge has dug in a little way, the broad plate offers considerable resistance to the sand, forcing it to go on digging instead of moving horizontally through the soil. The construction of the anchor-dredge with a single digging side leaves it clear from the obstruction of arms or chains and free to dig to its whole depth. The long single arm prevents the warp from disturbing any fast-moving burrowers which are about to be dug up, besides controlling the 'angle of attack' of the dredge. To keep the dredge the right way up, it was at first lowered on a double bridle one part of which fell off as soon as it reached the bottom. A much simpler method was subsequently devised. The short upright C (Fig. 1) was removed and a trawl-float lashed in its place. The float has sufficient buoyancy to keep the dredge the right way up as it is being lowered.

The first time the new dredge was tested in moderately deep water, on a ground of fine sand near the Eddystone, it collected a terrebellid polychaete, *Lysilla loveni* Malmgren, new to the Plymouth fauna. This result seemed to justify the belief that the dredge was more efficient on that type of ground than those which had previously been used.

The anchor-dredge is primarily intended simply as a collecting instrument, and not as a quantitative bottom-sampler. It might sometimes be used in conjunction with a bottom-sampler to ensure that the more deeply burrowing animals are not overlooked.

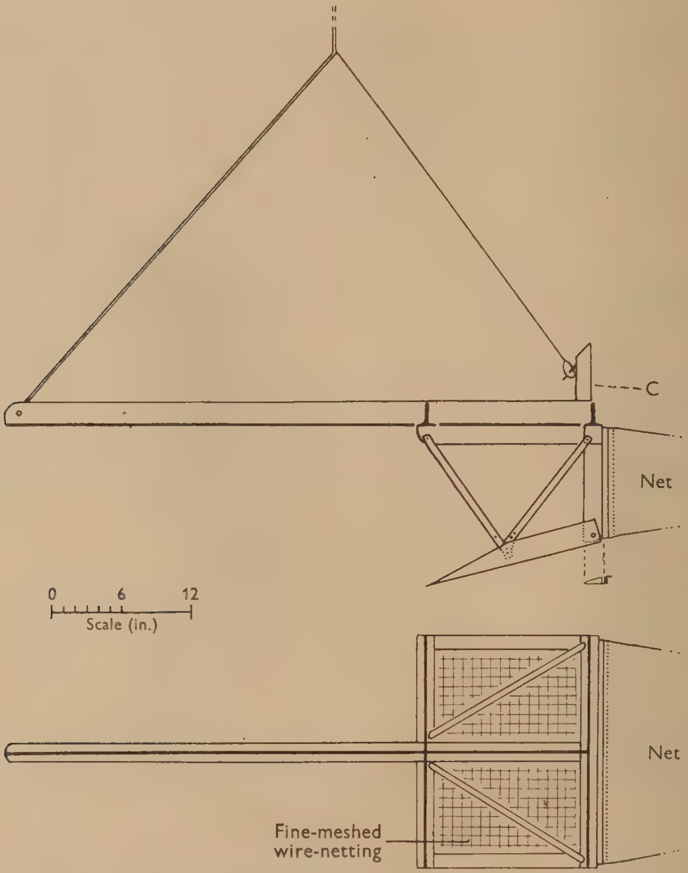

Fig. 1. Plan and side-view of the 'prototype' anchor-dredge.

STRUCTURAL DETAILS AND METHODS OF OPERATION

Fig. 1 shows the structural details of the first model of the anchor-dredge. It was built very cheaply from scrap material, but has proved satisfactory for testing the design. Although originally intended for the motor-launch *Gammarus*, it has also been worked from the M.F.V. *Sula*. Experience has shown that a slightly smaller model would be more suitable for the *Gammarus* because, without a power capstan, it is hard work for three men to haul the present dredge when the net is full. The weight of the dredge empty is

42 lb. (19 kg.) and it has dug up as much as three bathfuls of sand in one haul. The net may be of stramin or fine netting. For general purposes cotton 'lant' net of ½-in. mesh was found successful.

The best position for working the dredge from a trawler is through the forward gallows. While the ship is moving or drifting very slowly astern the warp is paid out as fast as possible. If the trawl-winch will not 'steam out' the warp, the use of rope is preferable, to avoid pulling the warp off the winch drum by hand. When the length of warp released is about twice the depth of the water the dredge will bite sufficiently to pull the warp off the winch by itself. The ship is then given a touch astern on the engine, and the warp allowed to run out freely until its length is at least three times the depth of the water. At this point a gradual application of the winch brake causes an increasing strain on the dredge, and eventually brings the ship to a stop. One such tug is all that is necessary to secure a good sample, and hauling may begin immediately the ship has lost sternway. As far as possible the dredge should be shot and hauled in a straight line; for, should the ship drift off this line, the lateral strain imposed on the long arm of the dredge might be too strong for it. This effect is more readily avoided by the use of the forward gallows, for then, as the dredge is hauled, the ship pulls herself ahead and can be readily controlled; whereas, if the dredge is worked from the stern gallows, the ship moves slowly astern during hauling and is consequently very difficult to steer. From a motor-launch a rope warp is normally used, and shooting the dredge is simplified. The boat can now be allowed to drift during hauling as the strain on the dredge is much less.

RESULTS

Both from inside and outside the Sound the anchor-dredge has taken a number of animals which had previously only been caught very rarely by other dredges or are normally only taken by digging on the shore, e.g. *Cerianthus lloydi* Gosse, *Myxicola infundibulum* (Renier), *Ensis siliqua* (L.), *Lutraria lutraria* (L.), *Acronida brachiata* (Montagu).

Comparison with other Dredges

In Whitsand Bay on a sandy bottom the *Sula's* heavy dredge, which has a slightly oval frame, could not catch any of the in-fauna except the small whelk *Nassarius reticulatus* (L.), which lies just buried, half an inch to one inch below the surface. Two hauls of the anchor-dredge, however, took five large lamellibranchs (two *Lutraria lutraria* and three *Callista chione* (L.)), four *Echinocardium cordatum* (Pennant) and several polychaetes.

The conical dredge is normally recommended for sampling the in-fauna (E. J. Allen and S. W. Kemp in Fowler & Allen, 1928, p. 329); but one underwater observation showed that, although the bag filled up when the dredge was towed some distance, it did not dig in more than approximately three inches. Moreover, if the towing speed was too great, the dredge turned on its cutting edge until the net pointed upwards and no digging at all was possible. The anchor-dredge dug to six inches with the *Gammarus*, and probably to about eight or nine inches with the *Sula*.

The anchor and conical dredges were compared with one another in the following test from the motor-launch *Gammarus*. At a depth of about 25 ft. of water in Cawsand Bay the dredges were each shot twice on either side of a marker buoy. The anchor-dredge in four hauls took eight complete and six damaged razor shells (*Ensis* spp.). The conical dredge in its four hauls also took six damaged *Ensis*, usually the top third of the shell, but not a single entire specimen. The conical dredge caught fifteen *Nassarius* sp. compared to the anchor-dredge's six, indicating that it had dragged for some distance before digging. This dragging could often be felt on the warp. To make the conical dredge dig it was necessary to stop the engine until the dredge had checked the boat's way, and then very gradually increase the speed of the engine. If the warp was slack as the speed of the engine was increased, giving the dredge a strong tug, it broke out of the ground immediately; unlike the anchor-dredge which breaks out only when the warp makes an angle of rather less than 45° to the vertical.

In a further test with the *Sula* in Whitsand Bay the following catches of large burrowing animals were made, as shown in Table I. The results are based on twelve hauls of the conical dredge, of which the two poorest have been omitted, and ten hauls of the anchor dredge including one in which it came up partly broken. As the conical dredge did not dig in sufficiently to stop the ship, it was dragged over a distance of at least 50 yards. The depth was approximately 6 fathoms and about 30 fathoms of warp were used for each dredge.

TABLE I

	Conical dredge	Anchor-dredge
Ensis spp. either whole or in part, but longer than 1½ in.	2	8, including one 7-in. *Ensis siliqua*
Callista chione (large bivalve)	3	14
Echinocardium spp.	5	34

Another dredge which also digs deeply into sand or mud has been described by G. Gustafson (1934). It was a circular or ring type with arms. A similar dredge was tried out at Plymouth before the war but was found too heavy for general use.

IMPROVEMENTS

Fig. 2 shows a design for a slightly enlarged and strengthened model of the anchor-dredge, which it is hoped will be suitable for working from either the *Sula* (length 60 ft.) or the *Sabella* (length 90 ft.). The base plate is

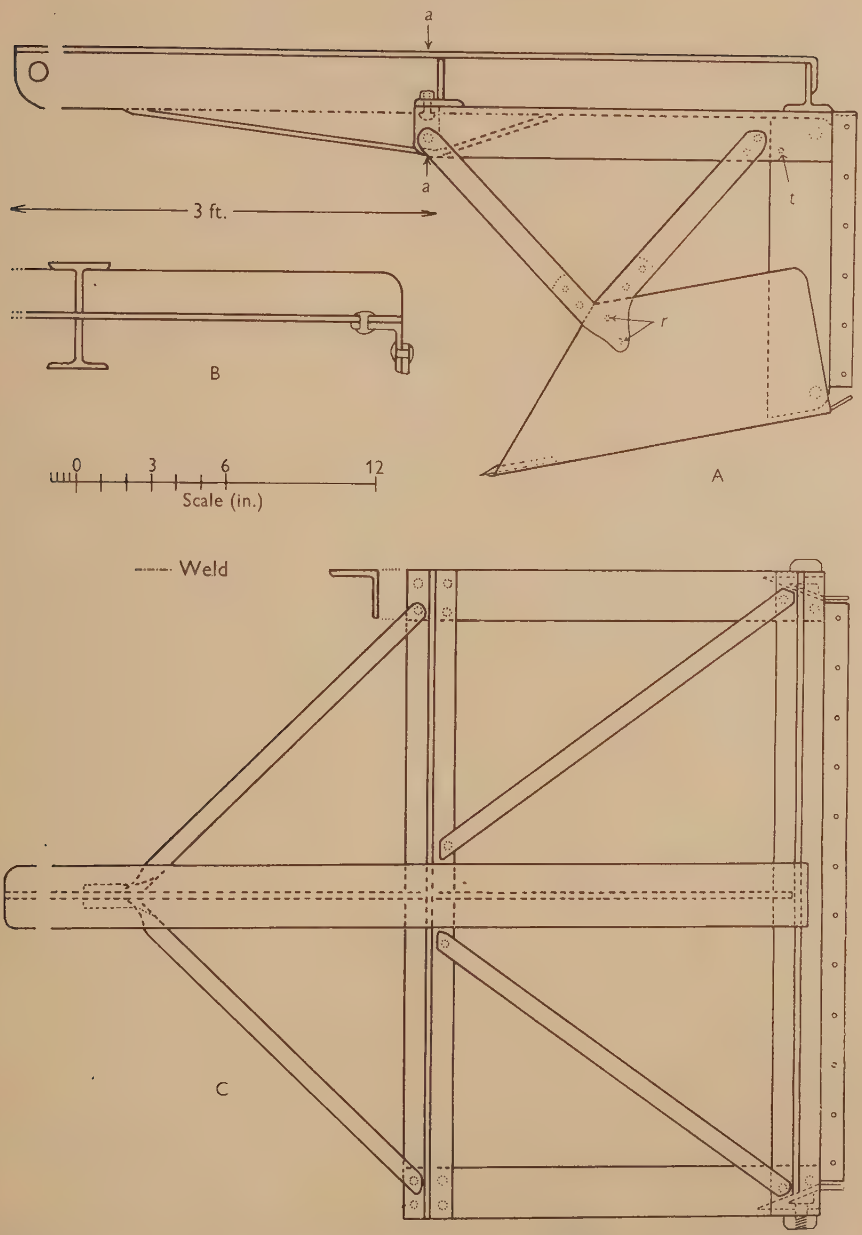
a
a
3 ft.
t
r
B
0
3
6
12
Scale (in.)
A
Weld
C

Fig. 2. Plans of the enlarged and strengthened anchor-dredge. For clarity some details have been omitted. A, view from the side. B, section through *a*...*a*. C, view from above. The function of *r* (small copper rivets) and *t* (a small bolt) is explained on p. 198.

thicker, $\frac{3}{16}$ in. instead of $\frac{1}{8}$ in., with large $\frac{1}{8}$ in. side plates to prevent loss of material. Should the base plate be caught under a boulder or other obstruction the two small copper rivets (*r*) shear, leaving the plate free to swivel. If the strain is very great, the small bolt (*t*) can also shear so that the whole dredge opens out. The long arm is heavier than in the first model, and has two additional T-pieces welded on to provide extra strength at its junction (*a*...*a*) with the first of the cross T-pieces. These change the arm to an I-section girder at this point, where the greater part of the lifting strain is carried.

I am most grateful to Captain W. J. Creese and the crews of the *Sula* and *Gammarus* for their help and cooperation in testing and working the dredge.

SUMMARY

The 'anchor-dredge' is a lightly built one-sided instrument designed to catch deep-burrowing animals. The depth to which it digs is dependent on the pull of the warp and not on the weight of the dredge.

From a trawler, it is best to work the dredge from the forward gallows, and give it a 'tug' as the ship is moving astern.

Comparative tests and direct underwater observations have shown that the anchor-dredge samples the in-fauna more efficiently than do either large rectangular and oval dredges, or the conical dredge.

A description and plan is given of a larger model, designed for use from a 60-ft. or 90-ft. ship.

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NOTES FROM THE PLYMOUTH AQUARIUM. II

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CONTENTS

	PAGE
The feeding of *Torpedo nobiliana* Bonaparte	199
An alga disliked by *Mugil chelo* Cuvier	203
Behaviour of *Gadus morrhua* L.	203
Cepola rubescens L. in captivity	204
Polyprion americanum (Schneider) in captivity	207

The purpose of these occasional notes has already been explained (Wilson, 1949). The present notes comprise a number of observations on the habits of fishes.

THE FEEDING OF *TORPEDO NOBILIANA* BONAPARTE

As known from ancient times, Electric Rays (*Torpedo*) have the power of giving severe numbing shocks, and it has repeatedly been stated that this power, which is electrical, is used both as a means of protection and for paralysing prey. That the shock is used in the capture of prey seems never to have been definitely proved. Indeed Roule (1935, p. 161) expresses the opinion that the electric shock is not used for securing food and even doubts that it is normally and effectively used as a means of defence. There appears to be no detailed account of the manner in which *Torpedo* feeds, but the ninth edition (1925) of the *Guide to the Aquarium of the Zoological Station at Naples* mentions (p. 106) that the Electric Ray rises from the mud to throw itself against approaching mullet (*Mugil*), and that the mullet, overcome by fright and electric shock, fall to the ground to be eaten. This very short and incomplete account was not seen until after the following paragraphs had been written.

Both *Torpedo nobiliana* Bonaparte and *T. torpedo* L. occur from time to time off Plymouth, the former being by far the commoner, and both have been kept in our aquarium tanks. They are sluggish fishes, spending most of the time lying still on the bottom, occasionally swimming for short spells. They swim by sculling with the tail, which has a large caudal and two dorsal fins. This mode of swimming differs strikingly from that of normal rays, which undulate the pectoral fins and trail their tails; it resembles the swimming movements of *Rhina squatina* (L.), another fish with a sluggish disposition and, as noted below, some other resemblances to *Torpedo*.

In the 1930's some time was spent observing *Torpedo* in the hope of discovering the manner in which the prey is captured. The rays observed never attempted to secure squid or dead fish thrown into the tank; but round fishes, such as cod (*Gadus morrhua* L.) and pollack (*G. pollachius* L.), if kept in the same tank, gradually disappeared one by one. In March 1935 a *Torpedo nobiliana*, 2½ to 3 ft. long, was seen with the tail of a cod, about 20 in. long, sticking out of its mouth. The cod was really too big for the ray and took over an hour to disappear. For several days afterwards the ray had a bloated appearance. Later in the same year I arrived at the tank in time to see a pollack disappearing into the mouth of the same or a similar ray. Late in 1937 another *T. nobiliana* almost certainly killed a pollack much too big for it to swallow. The pollack was found lying on the bottom of the tank still twitching. Round its middle was a dark mark, and dissection revealed that the vertebral column was broken. However, in spite of much time spent in observation, the manner of attack was never seen.

When the aquarium was reopened after the war further specimens of *Torpedo* were obtained, but in general they were unwelcome owing to the apparent impossibility of feeding them except with living fish. The same difficulty had occurred with *Rhina squatina*, but had been overcome by drawing dead fish through the water on the end of a wire. When the fish arrived near its mouth, the *Rhina* gave a very quick upward thrust of the head and rose from the ground to seize it. In *Rhina* the large mouth is at the extreme anterior end and is well suited to this habit. It was thought that *Torpedo* might similarly react to a dead fish drawn smartly through the water to within striking distance, though how it would attack it was a matter for speculation. The mouth of *Torpedo* is relatively much smaller than that of *Rhina* and is situated underneath the head well behind the anterior end, a poor position, apparently, for seizing actively swimming prey.

In the autumn of 1952, when two medium-sized *Torpedo nobiliana* (each about 2 ft. long, one a male, the other a female) had settled down in the largest tank, the feeding technique used for *Rhina* was tried and at once proved successful. A dead pout, *Gadus luscus* L., on the end of a wire fixed to a bamboo pole, was pounced upon by the male ray as soon as it was drawn to a position a few inches above the bottom in front of the ray. With a very quick movement the ray sprang forwards and upwards and made to envelop the fish, its wings (pectoral fins) and snout (the straight anterior border of the head) being curled around ventrally. The stiff wire prevented the completion of the manoeuvre, and as the pout was pulled away and upwards the ray adopted first a vertical position, with the wings surrounding the prey, and finally turned over upside-down in mid-water. It could then be seen that the pout was between the jaws of the ray, whose wings, snout, pelvics and tail were all bent or bunched upwards (that is ventrally) as if to surround it. The pout was unhooked from the wire, and the ray righted itself and flattened itself out on the bottom, by which time the meal had been swallowed. Much the same performance was repeated with another pout, after which the ray lost interest and on that day gave no further display.

The female was then tempted. It pounced in a similar manner, but, encountering obstructing rockwork, failed to secure the fish. In spite of repeated tempting no further interest in trailed fish was shown that day.

Subsequently many more pouncing responses were elicited and the manner in which *Torpedo* normally captures its prey became clear. The ray lies perfectly still on the bottom until a fish swims within reach, that is into a position just in front of the ray and not too far above the bottom. For a ray 2 ft. long the maximum distance above bottom seems to be about 6 in. The fish can approach from any direction. On one occasion a ray pounced on a bamboo pole as it was pushed quickly downwards to retrieve a detached bait lying motionless on the bottom. The bait must not be moved too slowly, otherwise a response does not seem to be forthcoming. The experience with the bamboo pole suggests, too, that detailed shape is unimportant. The bait need not touch the ray. Baits used were pout, hake and horse mackerel. The baits were attached directly to a wire, or to a nylon thread attached to the end of the wire. Baits on nylon threads were completely covered over and pinned to the bottom by the pouncing ray, the wings and anterior border of the head being bent downwards to entrap it. This pinning of the prey to the bottom is a normal procedure for ordinary rays; those which feed on crustaceans such as small crabs swim over them and pin them down until the jaws are able to take hold. Chewing movements are then visible on the upper surface above the gill arch region, and such chewing movements were sometimes seen when *Torpedo* had a fish below it. Strangely enough, *Torpedo* never succeeded in taking hold of a dead fish lying motionless under it, however long it was left there, and the fish was invariably easily pulled out from under the ray. The only fish which were secured and eaten were on wires. A possible explanation is that the *Torpedo* missed the struggles or other movements of living prey, and that the slight prodding which must occur with a dead fish on a stiff wire held in the hand simulated those movements and enabled the ray to find it by the feel. Even so, although the rays readily pounced, sometimes repeatedly, on a fish trailed on a wire it was not often that they succeeded in getting it into the mouth and eating it.

The feeding reactions of *Torpedo*, so far as they appear to the eye, are not very different from those of ordinary rays in the Plymouth tanks, except that they are quicker and appear to depend less on the olfactory sense. Large *Raia clavata* are known to feed on fish such as herrings and sprats (Steven, 1947), but on the whole the food of ordinary rays consists of crustaceans, smaller and less able to escape quickly. *Torpedo* catches relatively large active fishes, and it seems a reasonable conclusion that it is able to do so because it can numb and quieten them with its electric organs. But before this conclusion can be accepted the giving of the shock during capture needs to be demonstrated.

A dead horse mackerel, *Caranx trachurus* (L.), about 9 in. long, was fixed to the end of a waterproof electric flex. Each wire of the double flex was soldered to an electrode and the electrodes were inserted into the muscular tissues of the fish, one at each end. The flex was connected to a Pye Scalamp Galvanometer. The fish was now trailed in the usual way. Several times the fish touched the back of the ray but no deflexion of the galvanometer occurred. Eventually the ray (the female) pounced, and at that instant when it folded its wings and head on to the prey a strong shock was registered. The ray failed to secure the fish. After several more attempts without any response the female was abandoned and the male was tried. As soon as the bait arrived in the proper position the ray pounced and again a strong shock was registered. This time the bait was secured and the ray began to swallow it with the flex still firmly attached. To release the flex by pulling out the electrodes the ray had to be drawn vertically up in the water and almost over on to its back, and up towards the surface, its wings and snout being folded ventrally as usual. It was some little time before the electrodes were tugged away, just as the tail of the horse mackerel was disappearing into the mouth of the ray, but no further shock was registered. The shocks given by both rays were probably considerable. The galvanometer, on lowest sensitivity, gave far more than full-scale deflexion, indicating that the shock was of the order of volts. With the instrument used it was not possible to obtain a better measurement; the purpose had been only to demonstrate that a shock is given at the moment the ray touches its prey.

The effect on a living fish may perhaps be surmised from an occasion when a turbot attempted to seize the bait at the moment the *Torpedo* pounced. The *Torpedo* missed the bait and partially enveloped the turbot in a vigorous manner, pinning it to the ground. Convulsive contractions of the electric-organ region (these are mentioned by Day, 1880–84) and strong chewing movements were seen. The tail of the turbot projected from under the ray at one side and curled stiffly upwards, and was not flapping about as might be expected of a trapped but otherwise unharmed turbot. After a time the chewing motions ceased but the ray still lay over the turbot. Eventually the ray was pushed aside with a pole, revealing the turbot, its tail now back on the ground, alive and undamaged but breathing perhaps a little slowly. After a few minutes it swam away, and it is still alive. The *Torpedo* gave no further responses that day. It is well known that after use the power of the electric organs is weakened and time is needed for recuperation.

During these observations the use by *Torpedo* of its electric powers in a defensive manner was also seen. On several occasions a dogfish or nurse-hound nosing over the ground for food came into contact with a *Torpedo* and on doing so gave a sudden start and swam away. Contractions of the electric-organ region were not then specially noticed. Conger eels nosing a ray have suddenly come to an abrupt stop and then quickly retreated backwards a long way. On two separate occasions large lobsters walking over the ray towards a piece of food suddenly jumped backwards with a vigorous flap of the tail.

At other times lobsters have walked unharmed over the rays. Once a *Torpedo* and a large *Rhina* lay facing one another a short distance apart. On a fish being trailed between them both attempted to seize it at the same instant, with the result that they collided head-on in mid-water. At that moment the *Rhina* gave a sudden start and swam away, displaying unusual energy for its species. To the onlookers there was no doubt that it had received a shock, probably the one intended for the prey.

The observations recorded throw little or no light on the manner in which the prey is perceived. The eyes of *Torpedo*, like those of *Rhina*, are small, but they may be sufficiently sensitive to detect the movement of an object of approximately the size and shape of a swimming fish suitable for food, and to fix its position. In nature all such objects are likely to be suitable for food and not a source of danger. Bateson (1890) figures the eyes of both *Torpedo* and *Rhina* and shows that while by day their pupils are only narrow slits, by night they are circular and widely open. However, the prey may be perceived by other senses, by the disturbance it makes in the water, or in other ways. The point demands investigation.

In these observations I have been much assisted by Mr G. R. Forster, and by the attendants Messrs W. H. Gladwell and A. N. Bennett. I am indebted to Mr B. C. Abbott for lending the galvanometer, and Messrs F. J. Warren and A. E. Stoate for working it.

An Alga Disliked by *MUGIL CHELO* Cuvier

In a tank containing grey mullet (*Mugil chelo* Cuvier) any algal growth on sides and rock is usually kept very short by their browsing activities. Consequently, when in 1950 an isolated growth of a small red seaweed on the top of a rock arch stood out conspicuously amid the eaten-down growths all around, the event was specially noted. Soon patches appeared in other parts of the tank and were obviously being left alone by the mullet. In April 1951 the alga covered fair areas of rockwork and of the sides of the tank and overflow pipe. In the same month the alga was identified by Dr M. W. Parke as *Polysiphonia urceolata* (Dillw.) Grev. in fruiting condition bearing tetrasporangia. A year later this small red seaweed covered almost the whole of the rockwork, and most of the sides of the tank and the overflow pipe. Several samples were taken and the previous identification checked. On this occasion the plants had vorticellids and other organisms growing on them, but the main bulk of the samples consisted of *Polysiphonia urceolata*, which can therefore be retarded as being distasteful to grey mullet.

Behaviour of *GADUS MORRHUA* L.

In December 1947 several evenings were spent photographing three large cod (*Gadus morrhua* L.) in the second-biggest tank in the aquarium ($15\frac{3}{4} \times 9 \times 4\frac{1}{4}$ ft. deep). One of the cod was blind in the right eye and it invariably took up a position in the top left-hand corner of the tank with its blind side close to the glass; from this position the good eye could survey the whole interior of the tank. The other two fish generally swam about together, keeping mainly to the other half of the tank. If together or singly they reached its neighbourhood, the blind-eyed fish immediately rushed at them and chased them away, returning to its corner afterwards. This blind-eyed cod died in March 1949 and proved to be female with maturing but not ripe ovaries. It was 71 cm. long.

The two companionable cod rarely separated for long and one would frequently rub its side against the other, often retiring a short distance to swim quickly back again and, swerving laterally, graze the lower middle region of its body against the other fish as it rushed past. The behaviour of these two strongly suggested courtship. It was always the same fish which followed the other about and took the active part in these proceedings. This fish died in October 1949; it was a male, 72 cm. long, with, at the time of death, immature testes. Its companion, which was so attractive to it, expired some weeks after the evening observations here recorded. It had very swollen ovaries and may have died from egg-binding. The length was not recorded but was close to that of the male.

Attempts to photograph the courtship were unsuccessful. The actions took place quickly at intervals, always well back in the tank almost out of range of the flashlights.

CEPOLA RUBESCENS L. IN CAPTIVITY

Day (1880–84) and Fage (1918) express the opinion that *Cepola rubescens* L., commonly called the Red Bandfish, prefers rocky situations at moderate depths, but at Plymouth this is not so. *Cepola* is most frequently taken in the trawl on, or near, the Rame Mud, a few miles seawards from Rame Head, at depths round about 25 fathoms, and it is rarely taken on other grounds. Specimens rarely survive capture; distension of the air-bladder caused the fishes to float at the surface when placed in a tank and death usually followed within a few hours. Since November 1950, however, a few individuals, taken in trawl hauls of short duration and raised more slowly than usual to the sea surface, have survived. A few have lived for some months in the aquarium, though not all together at the same time. The first of these, a *Cepola* 43 cm. long, was brought in on 23 November 1950 and lived until 26 June 1951, when it was attacked and partly swallowed by a very hungry three-bearded rockling (*Onos tricirratus* (Bloch)), which had been living in the same tank for the whole of that period. Other specimens did not survive so long, dying from various causes. One or two kept for 9 or 10 weeks were never seen to feed. Another fish got itself jammed between the edge of the glass and the wall of the tank; another was killed by a lemon dab (*Pleuronectes microcephalus* Donovan) which pinned it down, perhaps accidentally, under its body; some died without obvious cause. Those which survived for more than a few days were a small proportion of those brought in alive. The species thus seems to be readily injured, both in the trawl and in simple accidents during captivity.

In captivity a red bandfish usually takes some time to learn to feed. At first it may catch a few *Hemimysis lamornae* (Crouch), which breed in the tanks, but sooner or later it generally learns to take small pieces of squid (*Loligo*) or worms (*Nereis*) sinking down towards it from above, though for many days it may 'blow' such pieces out of its way. Once it is feeding it will compete for food with other fishes, and some individuals have even learnt to take squid from the hand. Towards other fishes *Cepola* can be fierce, biting viciously with long sharp teeth at small pollack and soles. A large *Cepola* was seen to snap at a smaller one.

Cepola swims slowly, with very pronounced body waves. The tank in which the specimens were kept was probably too small to encourage speedy swimming, but forward darts after food, followed by rapid backward retreats, were often seen at feeding time. While a few individuals have swum, or rested, horizontally close to the bottom the majority have maintained themselves vertically head uppermost, swimming with slow body waves, with the ventral surface close up against the slate sides or back of the tank, but never against the glass. This position may have been adopted to avoid the soles living on the bottom in the same tank; but it seems more probable that the vertical posture is normal. However, the individuals which by day always adopted such a position were occasionally seen, especially at night, after sudden switching on of the light, to be swimming slowly over, or resting horizontally on, the bottom. A variation in posture sometimes adopted for short periods has been to curl the posterior third, or half, of the body into a loop, and to rest with the loop on the ground, the anterior part of the body being upright. Sometimes a length of tail has been laid on its side flat along the ground with the rest of the body raised up off the bottom at an angle of about 30°, but bent or twisted to bring the front portion and head into the normal dorsum-upper-most position.

The vertical swimming habit in such an elongated tapering fish suggested to Mr P. G. Corbin that the fish may normally inhabit a vertical burrow, either of its own construction or one occupied, or abandoned, by some burrowing animal such as *Upogebia*, which is numerous where *Cepola* commonly occurs. Indeed, Mr Corbin informs me that more than once a *Cepola* has been brought up in a dredge full of mud and burrowing bottom fauna. With this possibility in mind vulcanite pipes of various diameters and lengths were put into the tank, horizontally, vertically and at various angles, to see if the *Cepola* would enter them, but none did so.[1]

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[1] On 24 February 1953, while this paper was in proof, a *Cepola*, length about 20 in., was obtained in unusually good condition. It swam vertically, as usual, and four days later was found to be inside a long vulcanite pipe of about $3\frac{1}{2}$ in. internal diameter. This pipe (which was suspended vertically with its lower end just above the tank floor in one corner, its upper end above water level) was receiving water from an inflow nozzle.

The surmise that *Cepola* normally lives in a burrow received support when one was several times seen, in July 1951, to take up a mouthful of the shell gravel covering the floor of its tank and blow it out again. Later, in February 1952, another specimen made determined efforts to excavate a hole and it got as far as making a well-defined depression in the gravel. Had the gravel been deeper, and had the sides of the hole not slid in as the bottom was excavated, the hole would undoubtedly have been dug out to a fair depth. Twenty or thirty times, after this fish was first observed to be busy, a mouthful of gravel was removed from the hole and deposited well to one side. On each occasion the *Cepola* approached the hole swimming horizontally over the ground, bent down its head vertically into the hole, seized a mouthful of gravel, straightened up and swam a short distance to where the gravel was dropped out of its mouth, and returned for another load. Now and again it rested upright on coiled tail, the loop of the tail within the shallow depression which was the hole it had made. In a stiff mud, such as that of the region where most *Cepola* are caught, the actions just described would excavate a vertical burrow which if carried deep enough would accommodate the fish. If it does live in such a burrow it may have its head near the entrance ready to dart out at passing prey. The fact that specimens which swam vertically by day were sometimes seen in a horizontal position at night may indicate that during the hours of darkness they naturally come out of their burrows to feed. Those that are caught in the trawl by day may also be out hunting for food. However, it is not yet established that *Cepola* ever lives in a burrow, and further observations are needed to confirm or refute this idea. Another possibility is that the excavating activity is connected with nest-building, but this seems unlikely, as the available evidence, although incomplete, indicates that the egg is pelagic (Holt, 1891). Moreover, Clark (1920) states that the spawning period is in August, whereas the most intense excavating activity was observed in February.

Since the above account was written further observations have been made. On 7 January 1953 several young *Cepola*, 5–10 cm. long, were caught in an Agassiz trawl 6½ miles south of the Breakwater, on a bottom of slightly muddy sand. When brought in they were floating with distended air-bladders, but the most lively of them were placed in one of the central table tanks in the aquarium and by the next morning a few of these were swimming normally. About noon Mr P. G. Corbin called my attention to one which was burrowing in sand beside a perforated vulcanite partition whose lower edge rested on the sand. The burrow was being constructed partially under this partition, the fish picking up in its mouth sand from the bottom of its hole, turning round and blowing it away well to one side. Relative to the size of the fish the depth of sand was sufficient for the excavation of a hole, as wide as it was deep, into which the fish was able to retire with its tail coiled around inside and its head out of the entrance. In a corner of the tank another small *Cepola* had excavated a shallower depression, but it and several others were swimming vertically in the corners or against the slate sides of the tank. Throughout the day they so continued, and the fish in its hole remained there. The following morning the latter and all but two of the others were missing. A search discovered a small *Cottus bubalis* Euphrasén hiding under a rock. When killed and opened the partially digested remains of at least two *Cepola* were found in its stomach.

The burrowing of these very young *Cepola* disposes of the idea that only breeding individuals do so, in order to make nests, and short of final proof strongly supports the supposition that *Cepola* normally lives in a hole or a burrow which it makes for itself. The form of the burrow has still to be determined, whether long and deep to take a fish stretched at full length, or shallow and wide in which it could lie coiled up. Perhaps both types are made, according to the depth and consistency of the bottom deposit. It may be that they are often excavated to pass beneath a stone or other solid object lying on or embedded in the surface of the deposit, in a similar way to the hole made under the partition.

POLYPRION AMERICANUM (SCHNEIDER) IN CAPTIVITY

A fine Wreck-fish or Stone-bass, *Polyprion americanum* (Schneider), about 50 cm. long, was caught, on 7 September 1951, about 5 miles south-west of Bolt Tail by Mr F. Jarvis, Hope Cove, Kingsbridge, and brought to the Laboratory, where it was placed in a tank containing various species of Labridae. The fish had been found inside a tea-chest which was floating on its side; there were no barnacles or other obvious growths on the tea-chest, according to information obtained from Mr Jarvis. Couch (1862) states that the species is especially prone to gather under masses of floating wreckage where it feeds on smaller fishes attracted to the shelter of suspended barnacles or weeds.

This specimen, probably the first of its species to be kept alive at Plymouth, soon settled down and since its arrival has grown about 5 cm. It has fed mainly on fresh fish, readily taking whole pout, *Gadus luscus* L., and small whiting, *Gadus merlangus* L. It will not eat squid—which most of the other fishes take so eagerly—unless it is very hungry. It has a fierce disposition which it wreaks principally on the largest ballan wrasses (*Labrus bergylta* Ascanius), leaving alone the smaller species, the gaudily coloured Cuckoo Wrasses (*Labrus mixtus* Kröyer), and even the smaller ballans. Almost whenever the large ballan wrasses, 30–40 cm. long, appear out of hiding amid the rocks, they are chased and often bitten. In August 1952, Mr W. H. Gladwell, the aquarium attendant, saw the *Polyprion* kill a large ballan wrasse by repeatedly pushing or beating it against the slate wall of the tank, the wrasse being held by the back. These large wrasses are too big to be eaten; and the *Polyprion* is scarcely likely to attack them because it is hungry, for it could more easily catch and eat the smaller wrasses. Indeed, the *Polyprion* attacks the large fishes as readily after a full meal as before. It is apparently the sight of another large fish which arouses the animosity of the *Polyprion*, which may well have a habit of attacking other fish near its own size, or larger, which appear to be encroaching on its territory. As a fish-eater it would tolerate the presence of fishes of a size suitable for food.

When not attacking another fish the *Polyprion* swims slowly up and down its tank in mid-water and does not frequent the rocks as do the wrasses. Forward propulsion is aided by movements of the enlarged lobes of posterior dorsal and anal fins; the spiny anterior or dorsal is erected as the fish turns. It is often attracted by and watches closely anyone looking into its tank. The overall colour is a fine steely-blue with a velvety plum-like bloom, lighter on the underparts. The caudal fin is bordered by a dark band, with a tendency to a light outer edge. The iris is golden and black and the tip of the protuberant lower jaw has a dark patch. Altogether it is a strikingly handsome fish.

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THE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY LARVAE

THE 1952 EXPERIMENTS

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INTRODUCTION

In respect of their influence on the settlement reactions of *Ophelia bicornis* larvae, sands can be classed as attractive, neutral, or repellent (Wilson, 1953: mainly on the basis of the 1951 experiments). There are varying degrees of attractiveness and repellence, and the relative effect of sands on larval reactions can be tabulated. From the tables 'it should be possible to forecast with fair accuracy the settlements which will be obtained in mixtures of various sands' (Wilson, 1953, p. 427). Part of the 1952 experiments were devoted to investigating this possibility.

Earlier experiments had indicated, though not finally proved, that some of the repellent property of Salthouse Lake sand could be transferred, by contact under water, to Bullhill Bank sand. This was demonstrated by mixing large grains of the latter with small grains of the former and separating by sieving. In the first experiments along these lines (Wilson, 1952) the sands were not fresh and were sometimes dried for sieving, and all were subsequently sterilized. In 1951 a similar experiment (43 A) with fresh sands, mixed in sea water, again seemed to show partial transference of a repellent factor, presumably organic in nature. The technique used, however, was not entirely satisfactory, for it involved interference with the natural grade of the sand. Also the sand whose properties it was intended to alter was not itself completely free from organic impurities. In the 1952 experiments these objections were met by using acid-cleaned sands of normal grade sewn into fine-meshed bolting-silk envelopes. The envelopes were partially buried in the fresh natural sands from which it was hoped to abstract the repellent factor, and possibly also an attractive one.

A suspicion that these factors included one or more substances of organic nature led to some tests with sands incubated in nutritive media. In this connexion I am much indebted to Dr C. P. Spencer, not only for practical assistance with the incubation but also for much stimulating discussion on the whole problem involved. The incubation experiments in the present series are largely the result of his suggestions.

The 1952 experiments cover also a number of other points, as will be apparent below. It was not practical during the short breeding season to investigate only one aspect at a time, and each experiment was compounded, as usual, with more than one object in view. To enable a larger number of tests than before to be dealt with in one day, actual counts of the settled and settling larvae were avoided as much as possible. The assessment of the relative attractiveness of the sand samples had to be speeded up, for it had become necessary to test most sands both by the free-choice method and by the conical vessel method. The former method alone does not distinguish between neutral and repellent sands; the latter distinguishes imperfectly between neutral and attractive sands. Therefore both methods are required. The maximum number of samples which can be dealt with in one day if the larvae be counted, as hitherto, is about twelve. For many results, however, exact counts are not necessary, obvious differences being apparent on inspection. In a few minutes such differences can be recorded in words, when to obtain figures would take much longer. Only when the differences are not great are words unsatisfactory, but in the type of experiments used in this work close figures are also of little significance. By drawing up a scale of impressions of increasing number, expressed in words, three times as many sand samples could be disposed of in one day as when counts were made. The scale of words is shown in Table II and is used throughout Tables III–XI. When two samples are shown to differ one from another only by adjacent words in Table II, then the difference between them is scarcely significant, but if the words are far apart then the difference is real. By spreading out all the sand samples, of any one experiment, in dishes alongside one another it was relatively simple to compare them for larval content, and grade them accordingly.

NOTES ON TECHNIQUE

General techniques differed little from those of previous years (Wilson, 1952, 1953). The flat-bottomed Pyrex crystallizing dishes (about 7 cm. diam.), in which little heaps of the sands were tested under free-choice conditions, were all cleaned in hot strong sulphuric acid. The conical vessels were cleaned only in hot water containing Lissapol C and scrubbed with a bottle brush, as were also the finger bowls in which the fertilizations were made. The earlier experiments (Exps. 52–56) were in water from off Millport in the Firth of Clyde, the later ones (Exps. 57–59) in a mixture of the Clyde water and water from the English Channel near the international hydrographical station E 1, or in E 1 water only (Exp. 60). All sea water was passed through a Berkefeld filter before use.

Sands which are stated to be acid-cleaned were first washed in fresh water and then heated in fuming concentrated sulphuric acid for at least 30 min., cooled and washed in glass-distilled water and stored in acid-cleaned bottles in distilled water. Bolting silks were cleaned in acetone. New pieces were always used and different pieces for each kind of sand. Bolting-silk envelopes were sewn with white cotton, also cleaned in acetone. These envelopes were made by folding the silk and sewing bent-over edges along three sides, sand being inserted before the last side was sewn up. They were flat and measured roughly 15 × 10 mm. The sand inside the envelopes spread out into a thin layer and was in close proximity to the sand on which the envelope lay and which was sprinkled over it. The dishes containing these envelopes were kept in a good light but shielded from the sun. Daily, as a rule, the envelopes were turned over with forceps sterilized in a bunsen flame, fresh sand being shifted over on top of them. To remove the sand inside they were cut open with sterilized scissors after all grains on the outside had been carefully shaken off. The silk of envelopes exposed to Salthouse Lake sand was often blackened, but not so that exposed to Bullhill Bank sand. Occasionally, in all dishes, the silks showed pink patches after several days.

The flat-bottomed dishes in which larvae had a free choice of the various sand offered to them in little heaps were, as usual, kept in the dark undisturbed during the two days allowed for settlement. The conical vessels were, as usual, surrounded by black paper so as to be lit only from above, and they were decanted after about 24 hr. to wash away all larvae which were not settling. All larvae washed away by the decanting and re-filling process were examined to make sure that none were metamorphosing. It was confirmed that sands could be much disturbed without washing away metamorphosed or metamorphosing larvae, which always clung tightly to the sand grains.

THE EXPERIMENTS

The 1952 results (Tables III–XI) are expressed as grades of abundance (Table II), with few exceptions. Only larvae actually in the sand samples are mentioned in these tables. The unsettled larvae decanted from the conical vessels are not of special interest; neither are those in the free-choice dishes which were still swimming, or were lightly attached to the surface film or to the clear glass bottom between the sand heaps. There were always many such larvae and they were specially numerous when all the heaps were of neutral or repellent sands. They were always unmetamorphosed, except for a few metamorphosed worms that occasionally crawled out of overcrowded heaps on to the glass between them. Sometimes in heaps of fresh Bullhill Bank sand so many larvae had settled and metamorphosed that their activities caused a spreading of the sand until the base of the heap was extended to almost twice its proper diameter.

Each experiment had a control not recorded in the tables. This was a small flat-bottomed dish, the bottom completely covered with fresh Bullhill Bank sand, placed in the dark with the free-choice dishes. Invariably most of the larvae put into this dish were found to have metamorphosed, few or none being on the surface film, or still swimming.

Unless stated to the contrary, the sands mentioned in the tables were fresh, unwashed and unsterilized. They had been collected on the dates mentioned and had been kept since collection in clean stoppered jars. The Bullhill Bank sands were all from the surface of the middle of the Bank at places where *O. bicornis* adults were abundant. The Salthouse Lake sands were all from Station II (see Wilson, 1952, pp. 59 and 61), where *Arenicola marina* and other species were plentiful (for list of species see Holme, 1949) but *Ophelia bicornis* absent. Table I gives the percentages by weight of the various mesh sizes of these two sands, for comparison with the sands of previous years. The washings, normal sterilization (heating in a glass vessel immersed in a bath of boiling water for a few minutes) and treatments with activated charcoal mentioned were all carried out in tap water, as had been usual. The mixtures were all made by measuring, in the stem of a pipette, equal volumes in water of the two sands used.

The experiments are numbered consecutively with those already published.

Experiment 52 (Table III)

Series A consists of tests of sands put into medium (0·5 % peptone and 0·2 % glucose in sea water) 3–4 hr. after collection and incubated in a dark oven at 22° C. for 6 days. One sample of Bullhill Bank sand was inoculated with a very few grains of Salthouse Lake sand. At the end of this period the medium, which had become either yellow (Bullhill Bank sand) or greenish grey (Salthouse Lake sand) with a strong odour, was poured off and the sands well washed with clean sea water before testing. Microscopic examination of portions of the washed sands showed large numbers of minute organisms sticking to the sand grains, or free and in motion in the interstices.

Table III shows that incubation of the sands lessened their attractiveness for the larvae, compared with the unincubated controls, both under free-choice conditions and in the conical vessels. Salthouse Lake sand, both without and with incubation, was less attractive than Bullhill Bank sand correspondingly treated. Inoculation of Bullhill Bank sand with grains from Salthouse Lake, thereby introducing bacteria and micro-organisms from the latter place, had no noticeable effect. The hypothesis that Salthouse Lake contains species, culturable in the medium, which are repulsive to the larvae and which are absent from the Bullhill Bank is unsupported by this inoculation experiment. The culturable species, or their metabolic products, normally present in the Bullhill Bank sand, are attractive, or at least not repulsive, in normal abundance. When more abundant they may possibly be offensive to the larvae.

Series B and *Series C* were tests of mixtures. In the first the mixing in equal proportions of a fully attractive sand with one that is neutral (see Wilson, 1953, pp. 425–7 and Table XIII) induced, as was expected, a good settlement under free-choice conditions and an even better settlement in a conical vessel.

The second mixture, of a fully attractive with a fully repellent sand (Wilson, 1953, Table XIV), produced very little settlement under either condition, this result again being the one anticipated. The repellent property of the sterilized Salthouse Lake sand had a stronger influence on larval reactions than the attractive property associated with Bullhill Bank sand.

Experiment 53 (Table IV)

For *Series A* small portions of the acid-cleaned sands removed from bolting-silk envelopes after 5 days in sea water, with or without contact with fresh unwashed sands, were examined microscopically. That which had been in sea water only showed some very minute organisms, which may have been bacteria, and was otherwise very clean. The other sands were also relatively clean but, in addition to what may have been bacteria, a number of ciliates, minute diatoms and other living plant cells were seen. They were more abundant in the sand which had been in contact with the Salthouse Lake sand than with that from the Bullhill Bank.

The result (Table IV), compared with the two controls, shows a marked increase in attractiveness of the acid-cleaned sand which had been amid fresh Bullhill Bank sand. That which had been with Salthouse Lake sand showed no such increase; instead there appears to have been a slight decrease in attractiveness, this being most marked in the conical vessel test.

Series B, *C* and *D* are further tests of mixtures. In the first the formalin-treated sand appears to have been a little repellent and not neutral as a similarly treated sample mentioned in a previous paper (Wilson, 1953, Table XIII). No doubt different samples vary a little in their properties. The mixture in this instance induced somewhat smaller settlements than had been expected on the basis of formalin-soaked sand being neutral, but this result is in agreement with one component of the mixture having a slightly repellent action. The second mixture was to test a suspicion, based on earlier results (Wilson, 1952, pp. 103 and 155), that boiling Bullhill Bank sand for a prolonged period in distilled water makes it a little less favourable than normal sterilization, and therefore on the repellent side of neutral. In this test the boiled sand acted as a neutral sand and the result is closely similar to that of Exp. 52 A with normally sterilized sand. Again, different samples could vary according to their condition before boiling. The third mixture of a fully attractive sand with that of a repellent sand converted to attractiveness by the use of activated charcoal gave, under both conditions, the heavy settlements which had been anticipated.

Experiment 54 (Table V)

This was devoted to testing a number of mixtures of sands whose relative powers to attract or repel were fairly well known. The settlements obtained in all four mixtures were much as expected. In *Series A* and *B* the mixing of neutral with fully repellent sands produced strongly repellent mixtures. If boiling the Salthouse Lake sand made it more repellent than normal sterilization the fact is not shown by these fifty-fifty mixtures. In *Series C* the mixture of a fully attractive sand with a neutral or nearly neutral sand (in Wilson, 1953, Table XV, the oolitic sand is perhaps placed too far towards the repellent side of neutral) was favourable to settlement, but was not fully attractive. In *Series* D a fully repellent with a fairly attractive sand gave a mixture which was repellent, though not fully repellent.

Experiment 55 (Table VI)

Part of this experiment was devoted to tests of acid-cleaned Bullhill Bank sand inoculated with a few grains of fresh Bullhill Bank sand and fresh Salthouse Lake sand and incubated at 22° C. in a series of media containing less nutrients than the medium used for Exp. 52A. Sea water was enriched with 2·5 mg. per litre ammonium/nitrogen and this was further enriched with (*a*) 2·5, (*b*) 5, and (*c*) 10 mg. per litre of glucose to give a series of increasing concentrations. The results, though interesting, are too indefinite to be worthy of detailed consideration. So far as they go they suggest that inoculation of the acid-cleaned sand by either of the fresh sands, followed by incubation, slightly increases its attractiveness, and this may vary a little according to the concentration of the medium and hence probably of the number of micro-organisms present.

The remainder of this experiment, here designated *Series A* and *B* in Table VI gave definite results. *Series A* followed closely on the lines of Exp. 53A, using this time acid-cleaned Salthouse Lake sand which was of finer grade than the acid-cleaned Bullhill Bank sand used before (see Table I). Once again the sand which had been amid fresh Bullhill Bank sand became distinctly attractive, whereas that which had lain amid fresh Salthouse Lake sand did not, showing little difference from the controls.

Series B is a test of another mixture. In a previous paper (Wilson, 1953) it is shown that soaking fresh Bullhill Bank sand in distilled water can destroy its attractiveness under free-choice conditions, though a good settlement can be obtained when it completely covers the bottom of the vessel containing the larvae. It was rated as a neutral sand. In the present test there was a small settlement in the free-choice dish, although the sand had soaked for 3 days in distilled water. This suggests that this particular sample was a little on the attractive side of neutral, but the settlements obtained in the mixture with fully attractive fresh Bullhill Bank sand were of the type associated with a mixture of the latter with a neutral, or nearly neutral sand. They were, indeed, what had been expected.

Experiment 56 (Table VII)

For *Series A* three conical flasks each containing a small quantity (barely sufficient to cover the bottom if spread out) of acid-cleaned Bullhill Bank sand in filtered sea water were exposed to light on a window bench shielded from the sun. To one of these flasks a few grains (less than 1 %) of fresh Bullhill Bank sand were added, and to another a similar quantity of fresh Salthouse Lake sand. The flasks were gently shaken almost every day for 10 days, the sand in each flask piling up into a small heap in the middle. On the tenth day small samples from each were removed to be tested with larvae in the usual manner. In addition, a few grains of fresh Bullhill Bank sand and of fresh Salthouse Lake sand were mixed with small samples of the control sand to which nothing had been added previously. These mixed samples served as additional controls intended to distinguish between the effect of adding a few grains of the fresh sands just before the test and adding a few grains several days previously.

The settlements obtained with these various sands are set out in Table VII. The addition of a few grains of fresh sand just before testing with larvae seems to have made but little difference, except perhaps that the Salthouse Lake grains may have improved it slightly. It seems certain, however, that sands to which fresh grains had been added 10 days before testing were more attractive than any of the controls, and that the Salthouse Lake grains had had the greater effect.

The original sands in their flasks on the window bench were kept for nearly another 2 weeks, but some more acid-cleaned sand was added (on 1 July 1952) to the control flask because insufficient for another experiment remained. The tests were then repeated in an identical manner as part of Exp. 57 but only in a free-choice dish. The results were similar to those of the present experiment. There was no further increase in attractiveness of those sands to which grains of fresh sand had originally been added but these sands were still the most attractive.

Series B duplicates *Series A* except that the flasks were kept in a dark cupboard instead of on a window bench, and were exposed to light only for a few moments daily while being shaken. Table VII shows that the resulting settlements obtained were closely similar to those in sands exposed to the light. This *Series B* was also repeated (with the same sands kept longer) at a later date (as part of Exp. 58—free-choice dish only) and like the repetition of *Series A* gave settlements which differed hardly at all from the earlier ones.

It is not clear what these results indicate. If the improvement, with time, after inoculation is due to multiplication or metabolism of micro-organisms, then the species involved do not depend on light. The failure of the attractive factor to increase beyond a certain point, even with further time, might be due to early exhaustion of necessary nutrient materials from the filtered sea water used. Other explanations are possible. For instance organic matter on the fresh grains which were added may take time to transfer to the acid-cleaned sand.

Exp. 56 included tests of two more mixtures. The first (*Series C*) is a mixture of two neutral, or almost neutral sands, and gave, as anticipated, a small settlement under free-choice conditions and a good one in a conical vessel. It should be compared with Exp. 54C where the same oolitic sand was mixed with an attractive sand. The second mixture (*Series D*) is similar to that tested in Exp. 52C, except that here the Salthouse Lake sand was dried after washing and before normal sterilization. There seems to be little difference, both are strongly repellent, but in this experiment the larvae, which were a day older than usual, were a little more ready to settle than they generally are. The result, however, is only slightly different from that expected. Settlements in a fifty-fifty mixture of a fully attractive with a fully repellent sand are likely to vary around the neutral point according to the condition and age of the larvae. The 5-day-old larvae of Exp. 52C found such a mixture rather repellent, the 6-day-old larvae of the present series found it somewhat attractive. The antagonism of the two factors, one attractive, the other repellent, is clearly demonstrated.

The larvae in the clean finger bowl which supplied the experiment showed a precocious tendency to metamorphose without sand of any sort. Thus they displayed an unusual early elongation of the body, loss of prototrochal cilia and elongation of the bristles of the third setiger. Some larvae metamorphosed on the clean glass unusually early. These larvae were markedly more advanced in their development than were those of the same age in the bowl which had supplied larvae to Exp. 55. Account should be taken of this feature in assessing the results of the experiment.

Experiment 57 (Table VIII)

On two previous occasions (Exps. 53 and 55) an attractive factor from fresh Bullhill Bank sand was transferred to acid-cleaned sands consisting largely of quartz particles. In *Series A* of this experiment oolitic sand, which is almost entirely calcareous with but very few quartz particles, was used in place of an acid-cleaned sand. No attempt was made to clean it. Three samples were sewn up in 100-mesh bolting-silk envelopes. One, the control, was placed in sea water only, the other two in sea water amid fresh Bullhill Bank and fresh Salthouse Lake sands respectively. These fresh sands were changed after 11 days when fresher samples were available. After a total of 16 days the sands were removed from the envelopes and agitated in sea water on sieves of 86-mesh bolting silk. This was to ensure the removal of any particles of the fresh sands which might have worked their way through the 100-mesh silk envelopes to become mixed with the oolitic sands inside. The control sand was, of course, similarly agitated on 86-mesh silk: it was then divided into three portions, to two of which a few grains of the Bullhill Bank and Salthouse Lake sands were added. The oolitic sand which had been amid Salthouse Lake sand had a grey tinge and its silk envelope was blackish, as was much of the Salthouse Lake sand itself.

The result is shown in Table VIII. The settlements in the free-choice dish are considered first. There was little difference between any of the controls; the addition of a few fresh grains had had little or no effect and in all three the settlement was small. There was a significantly better settlement in the sand which had been with the Bullhill Bank sand, though the numbers of larvae settling were not large. Most of the larvae in the dish had gathered on the surface film or were still swimming freely, or were on the glass of the bottom, unmetamorphosed. The oolitic sand had apparently been made only a little more attractive by contact with Bullhill Bank sand, but none the less this increase in attractiveness was definite. On the other hand, the oolitic grains which had been amid Salthouse Lake sand had failed to induce any larva to metamorphose.

In the results with conical vessels there is no distinction between the controls and the oolitic sand which had been with Bullhill Bank sand. All gave settlements characteristic of neutral, or near neutral, sands. Only in the oolitic sand which had been with Salthouse Lake sand was there a difference to be noted. Here there were markedly fewer metamorphosing or metamorphosed larvae than in any of the other sands and there were rather more unmetamorphosed larvae present: the sand, in fact, appears to have been slightly repellent.

In *Series B* a new type of experiment was undertaken. A quantity of fresh Bullhill Bank sand was thoroughly shaken up with about three times its own volume of filtered sea water. After allowing all heavy particles to settle some of the water was carefully poured off into a clean dish. This was then examined to make sure that no sand grains, not even the smallest, were present. Only a little silt was to be seen. A small quantity of acid-cleaned Bullhill Bank sand was then added and the dish stood in the light, shielded from the sun, for 16 days. In a similar manner water in which fresh Salthouse Lake (St. II) sand had been shaken was used to soak another small quantity of acid-cleaned Bullhill Bank sand. In the Salthouse Lake water silt was plentiful but no sand grains were present. A control sample of the acid-cleaned sand was soaked in filtered sea water in the same manner. The experiment also includes a control of the same acid-cleaned sand stored in distilled water.

Table VIII, recording the results, shows that the sand soaked in water in which Bullhill Bank sand had been shaken induced much better settlements than did any of the others. The sand from water in which Salthouse Lake sand had been shaken also showed an improvement over the control, but not so great. This improvement contrasts with the opposite effect observed when acid-cleaned or oolitic sands are buried amid the Salthouse Lake sand itself. The control sample soaked in clean filtered sea water seems to have been slightly more attractive than the sample stored in distilled water.

In order to make reasonably certain that the presence of a few small sand grains, which might pass through 100-mesh silk, would not significantly affect the results obtained with sand samples enclosed in bolting-silk envelopes buried amid other sands, a test was made, in *Series C*, of acid-cleaned Bullhill Bank sand to which small quantities of fresh sands were added. The amounts added were appreciably larger than any which might be expected to pass through the meshes of the silk, and were 1 % or less of the whole sample. Although the conical vessel control was accidentally lost the results so far as they go confirm previous findings (Exps. 56A, B and 57A) that any effect is likely to be small.

As already mentioned (p. 215), Exp. 57 included a re-trial of the sands used in Exp. 56A.

Experiment 58 (Table IX)

An attempt was made to transfer the attractive factor in fresh Bullhill Bank sand, and the repellent factor in fresh Salthouse Lake (St. II) sand, to some artificial substance of as pure a composition as possible. I am indebted to Mr F. A. J. Armstrong for suggesting fused alumina, a reagent prepared by the Thermal Syndicate Ltd. The label on the bottle gave as the batch analysis; alumina 99·7 %, carbon 0·0013 %, alkalies 0·09 %. The alumina is in the form of granular particles. By sieving through bolting silks it was possible to obtain a 'sand' similar in grade to that of the Bullhill Bank. The graded alumina (all of which was coarser than 86-mesh and most of it coarser than 60-mesh) was thoroughly washed in several changes of distilled water, to reduce yet further the soluble impurities and clean the large grains of adhering minute particles. The resulting 'sand' was clean and white. For *Series A* small samples were placed, in 100-mesh bolting-silk envelopes, amid fresh Bullhill Bank and Salthouse Lake sands, and there was the usual control in an envelope in sea water only. After 7 days the contents of the envelopes were removed and examined microscopically. No sand grains had penetrated the envelope which had been amid Bullhill Bank sand, but there were a very few quartz grains and some silt mixed with the alumina from the Salthouse Lake sand. Therefore all three samples were sieved separately, in filtered sea water, on 60-mesh silk about a third of each sample being lost through the meshes. This ensured the removal of all foreign sand grains and silt which had penetrated the 100-mesh silk. The sands were again examined to confirm this. No foreign grains among those of alumina could be seen, but various minute living organisms were observed on the grains, especially those which had been amid Salthouse Lake sand. The alumina grains which had been with the latter were tinged pale yellow.

The results indicate that an attractive factor from the Bullhill Bank sand had been transferred to the alumina, but they barely show any such transference of a repellent factor from the Salthouse Lake sand. They are not, however, completely negative in that respect, and taken in conjunction with earlier tests on similar lines (Exps. 53A, 55A and 57A) provide additional evidence, though slight, that such transference is possible.

In *Series B* some tests are made with activated charcoal. It is not at all clear how this reagent reacts with repellent sands to make them attractive. The grains of such sands are always darkened with adhering specks of the carbon which may do no more than stick to and cover up matter objectional to the larvae. The granular activated charcoal used in this experiment was the kind intended for gas absorption. The other kind was that used for decolorizing, the same batch having been used in all previous experiments with this substance. It is a very fine powder, and only a very small quantity of very small grains was obtained by washing away a large quantity of the still finer powder. There was sufficient for only a very small heap in the free-choice dish and not enough for a conical vessel as well. These very fine carbon particles attracted a large number of larvae, but the proportion to metamorphose was small, much smaller than amid the granular carbon or amid the same but still finer carbon particles mixed with the acid-cleaned sand. This suggests that while the activated charcoal is liked for its own sake the larvae require, among other factors, a substratum of reasonable solidity if metamorphosis is to proceed readily.

Exp. 58 also contained a re-trial of the sands used in Exp. 56B (see page 215).

Experiment 59 (Table X)

Some earlier tests, included in Exp. 55, had indicated that inoculation of an acid-cleaned sand with a few grains of a fresh sand, followed by incubation (in the dark at 22° C.) in a medium, increases slightly the attractiveness of the sand. The test for inoculation with fresh Bullhill Bank sand was repeated here, the concentration of medium which gave the best result in Exp. 55 being used. This was sea water enriched with 2·5 mg. per litre ammonium/nitrogen and 5 mg. per litre of glucose.

There was some difficulty in obtaining satisfactory larvae for this experiment. All the fertilizations intended for it failed and earlier fertilizations had to be used. The larvae put into the free-choice dish were 7 days old; those into the conical vessels were 6 days old and were from the fertilization which had supplied Exp. 58.

The result in the free-choice dish agrees with the earlier results that inoculation with fresh sand grains followed by incubation increases the attractiveness of the acid-cleaned sand. The incubated control which had not been inoculated was also made more attractive in comparison with some acid-cleaned sand which had been merely stored in distilled water until tested. Settlements in the conical vessels with younger larvae were all very similar and any slight distinctions are in line with the dish results.

Lack of suitable larvae prevented other tests, which had been planned, from being made.

Experiment 60 (Table XI)

In Exp. 51 (Wilson, 1953) an attempt to test the effect on settlement of different grades of fresh Bullhill Bank sand was only partially successful owing to the unusually slow rate of development of the particular culture of larvae used. The experiment is repeated here with a larger number of grades. These were obtained by sieving fresh Bullhill Bank sand through bolting silks of the usual mesh-sizes used in these investigations (see Wilson, 1952, p. 59), the sieving being done in filtered sea water. The ungraded control sand was agitated on 100-mesh silk in sea water for a time equivalent to that needed for sieving the other grades. Only a very few of the smallest grains were lost. The heaps in the free-choice dish were all of the same size and were much smaller than usual. Hence the unusually small number of larvae settling in even the most attractive grades. Conical vessel tests were not used in this experiment.

The result supports earlier conclusions that grade size is not without influence on settlement. The grade in which the greatest number of larvae settled is that which until about 1950 comprised about 50% of the surface sand of the Bullhill Bank (see Wilson, 1952, p. 59 and Table II, p. 127). The settlement in the control ungraded natural sand was closely similar to that amid grains of 60–86-mesh sizes, the sizes which now form the greater bulk of the surface sand of the Bullhill Bank (Table I), and therefore of the control. Relatively large, or very small, grains attracted very few larvae or none at all.

Other Experiments

A number of other experiments were attempted towards the end of the breeding season late in July, but all were spoilt by the condition of the larvae. The fertilizations made in the latter half of July appeared to be excellent for the first 2 or 3 days. Afterwards the larvae went prematurely to the bottom and many stuck together in small and large clusters. This clumping has been shown to take place most readily when the water is in some way unsuitable for healthy development (Wilson, 1951). These fertilizations during the latter half of July were made in water from hydrographical station E1 off Plymouth, after the Clyde water available earlier had all been used up. In spite of the condition of the larvae several experiments were set up. The results they gave were, as far as they went, in line with previous findings. Thus another attempt to transfer to oolitic sand the attractive factor from fresh Bullhill Bank sand seems to have been successful. Some tests based on filtering water in which fresh sands had been shaken gave promising, though imperfect, results. These are, however, sufficiently interesting to merit further attention at a later date.

ANALYSIS OF SANDS FOR NITROGEN CONTENT

Dr C. P. Spencer kindly undertook the analysis of surface samples of Bullhill Bank and Salthouse Lake (St. II) sands collected on 9 May 1952. The sands were first thoroughly washed in clean sea water, from the laboratory circulation, to remove silt. They were then sieved, in sea water, through 26-mesh bolting silk to remove large mineral particles and any larger living organisms which might be present. The Salthouse Lake sand was further sieved through 40-mesh bolting silk, for it was found that a few small molluscs and worms had passed the larger mesh. Samples of the sand were then digested with the digestion mixture of Harvey (1951) and distilled with a Markham micro-stream distillation apparatus (Markham, 1942). The figures given below are the mean of triplicate estimations. The blank estimations were done on separate samples: Bullhill Bank sand, 490 μg. N/g. wet weight; Salthouse Lake (St. II) sand, 1760 μg. N/g. wet weight.

In view of the thorough washing, the nitrogen found must have been present on the sand grains in an insoluble form and is therefore probably organically bound. If this assumption is made, the nitrogen determination estimates organic matter, and so the Salthouse Lake sand, at least after washing, contained some three times as much organic matter as that from the Bullhill Bank.

The striking effect on settlement of treating sands with activated charcoal has frequently been demonstrated, repellent sands being made attractive by its use. I am much indebted to Mr F. A. J. Armstrong for making analyses in an attempt to find out whether the activated charcoal removed organic nitrogen compounds from the sand. The attempt was unsuccessful because the high nitrogen content of the activated charcoal masked any possible changes in the nitrogen content of the sand.

Discussion

In assessing the results of experiments such as these, and especially when comparing one experiment with another, it is essential to remember that they are based on the reactions of living organisms of which no two are ever quite alike. There is much accumulated evidence, not always set out in these papers, to show that different cultures of larvae vary in the readiness with which they will metamorphose under relatively unfavourable conditions, such as those of a clean glass vessel without sand. Some cultures remain free-swimming, with no tendency to metamorphose, longer than others of the same age, or younger, even when beside them on the same table under, practically identical conditions of temperature and lighting. Thus quite apart from variation in the numbers of larvae used from experiment to experiment, the results are to some extent affected by the characteristics of the particular batch of larvae used, as well as by their age when this is different. But in any one experiment very seldom has more than one fertilization of larvae been used, and all comparisons are made between larvae of the same age and with the same mixture of characteristics. Within each experiment results are thus strictly comparable. These remarks are necessitated by the long series of experiments which have now been published and which if compared without these points in mind may occasionally appear to be contradictory. For instance, as pointed out above (p. 216), in Exp. 56 the particular larvae used showed a precocious tendency to metamorphose without sand of any sort. This may well explain why more of them settled in the acid-cleaned sand straight from storage in distilled water than was usual for larvae of their age. Though this peculiarity has to be remembered when comparing this particular experiment with any other, the comparisons between the different sands in the experiment are not in the least invalidated. On the whole, atypical results due to variable characteristics have been infrequent. Most results, indeed, have been consistently repeatable.

The results with mixtures may now be considered. Before the present series of experiments was started, a table was drawn up (using information gained in 1951) listing the mixtures it was proposed to test and forecasting the intensity of the settlements expected in them under both free-choice and conical vessel conditions. Twelve mixtures were proposed and, as already noted in the descriptions of the actual experiments, the results with them showed very little variation from those expected. Such small variations as occurred, chiefly in Exp. 53B and C, can be attributed to one component of each mixture not having been quite of the same neutral or repellent property as a similar sand previously assessed. Such variation between sands of different batches is only to be expected. The experiments, however, show that if the relative power to attract or repel of two sands be known then it is possible to forecast the intensity of settlement which will be given by 5-day-old larvae in a mixture of equal volumes. A mixture consisting of two attractive sands, or of an attractive with a neutral, remains attractive. A repellent sand with another repellent, or with a neutral, gives a repellent mixture. Neutral with neutral remains neutral. Attractive sands mixed with repellent sands produce results varying on either side of neutral in accordance with which factor, attractive or repellent, is dominant. Only when the opposing factors are equal will such a mixture be neutral.

That the attractive and repellent factors are distinct, and at least to some extent independent of the mineralogical characters of the sand grains which carry them, seems to be shown by several experiments in which they were transferred to sands consisting mainly of quartz grains (after drastic cleaning in hot concentrated sulphuric acid), to a mainly calcareous oolitic sand, and to a purely artificial sand of fused alumina. The attractive factor was transferred quite readily, though no sand so treated became nearly as strongly attractive as fresh Bullhill Bank sand itself. Before 1952 no experiment had shown transference of an attractive factor. Indeed the existence of such a factor was almost unsuspected before the results of the 1951 experiments had been considered. Evidently, the attractive factor is present only in fresh sand, particularly that from the Bullhill Bank, and that almost any treatment including sterilization, drying, subjection to hot concentrated sulphuric acid and red heat destroys it.

The repellent factor, on the other hand, is most readily demonstrated in sterilized or dried sand, particularly that from the Salthouse Lake (St. II), though hot concentrated sulphuric acid or red heat destroy it. Its transference, though imperfectly achieved both in earlier experiments and in 1952, seems to be no less real than the transference of the attractive factor. In 1952 its transference may have been complicated by the presence of the attractive factor, for both seem to be present on fresh Salthouse Lake (St. II) sand, which is normally almost neutral. Neutrality could result, as is shown by the mixture experiments, when the two factors are balanced against each other.

The manner in which transference takes place from fresh sands to acid-cleaned or other neutral sands is not clear. For the attractive factor, physical contact of the sand grains is evidently not necessary, since it was present in sea water in which fresh Bullhill Bank sand had been shaken (Exp. 57B). It seemed also to be present, though less concentrated, in water in which fresh Salthouse Lake (St. II) sand had been shaken (Exp. 57B). The latter experiment and certain inoculation experiments (Exps. 55, 56A and B) appear to show that the attractive factor is present on fresh Salthouse Lake sand and can be separated from the repellent factor which is also there. The repellent factor seems to be transferred less readily than the attractive factor, and then only to sands in close proximity if not in actual contact with it.

The experiments involving incubation of sands in media are difficult to interpret. In the first of these experiments (Exp. 52A), where fresh sands were put into a medium which produced a heavy growth of organisms, the sands became, even after thorough washing, less attractive than before. On the other hand, acid-cleaned sands inoculated with only a few grains of either of the fresh sands, and incubated in weaker media producing only a relatively small growth of organisms (Exps. 55 and 59), showed an increase in attractiveness after incubation. Even without enriching the sea water, and at room temperature both in the light and in the dark, the inoculation of acid-cleaned sand with a few grains of fresh sand of either sort, followed by a period during which a small growth could take place (Exp. 56A and B), resulted in a small increase in attractiveness. These experiments, taken by themselves, might indicate that the attractiveness or repellence of a sand depends not on two different factors but on the relative abundance of a single factor. It will be remembered that the organic nitrogen content of Salthouse Lake (St. II) sand was some three times that of Bullhill Bank sand (p. 221). None the less, other results are more easily interpreted if two factors are assumed, one of which, the attractive one, is readily destroyed by sterilization, drying and other simple treatments.

The natures of the attractive and repellent factors are still unknown, but on the evidence are probably organic or the products of organic activity. The results with sterilized sands suggest that the repellent factor may be non-living material; but the status of the attractive factor is even less evident.

Even when the nature of the factors is known the manner in which the larva perceives them, or is affected by them, has still to be sought. The experiments with activated charcoal may give a clue on how the attractive factor stimulates the larva to settle and metamorphose. This highly adsorbent substance appears to be attractive in itself (Exp. 58 B), although for maximum effect it must be present in particles of a size simulating a sand, or have sand grains mixed with it. The very property of adsorbing readily, by aiding the larva to get rid of excretions, may act favourably on the larva. The attractive factor might thus be something which removes excretory matter from the larva; though this is by no means the only possibility. It is well known that minute amounts of copper, or other substances, stimulate the larvae of certain species to metamorphose (see Wilson, 1952, pp. 51–2 for a brief discussion and references). The activated charcoal is not a pure substance and I am indebted to Mr F. A. J. Armstrong for showing that it contains an appreciable amount of copper. The decolorising charcoal, which has been used throughout these investigations, gave, on analysis, 320 parts per million of copper, while the granular charcoal used in Exp. 58 B gave 600 parts per million. From the natural sands themselves the amounts of copper extracted by heating to fuming with nitric and sulphuric acids were: Bullhill Bank, 6 parts per million; Salthouse Lake (St. II), 8 parts per million. The total copper contents, determined after opening the samples by hydrofluoric acid treatment, were 24 and 15 parts per million respectively. These figures are given without implying that they are of significance in relation to settlement in the natural sands; whether the larger quantities present in the charcoals influence metamorphosis cannot be decided on present evidence.

The experiments with activated charcoal again showed that if metamorphosis is to take place freely the particulate matter must be of a grade not too far removed from that of the natural sand itself. This is further brought out in Exp. 60 in which graded sizes of Bullhill Bank grains were tested under free-choice conditions. The grades which form the bulk of the surface sand of the Bullhill Bank attracted the largest settlements and there was a marked falling off in the numbers of larvae settling in coarser and finer grades. Sands can evidently be too fine or too coarse.

Much of the foregoing discussion is speculative, and further experiments will surely modify or refute some of the ideas put forward, but these ideas are of value in so far as they facilitate the planning of future work.

SUMMARY

Previous conclusions that it should be possible to forecast the intensity of settlements which will be obtained in fifty-fifty mixture of two sands, provided the relative attractiveness or repellence of each component is known,

were fully confirmed. The results of these mixture experiments agree with the classification of sands as attractive, neutral or repellent.

It is shown that an attractive factor is capable of transference, in sea water, from fresh Bullhill Bank sand to acid-cleaned quartz sands, to calcareous oolitic sand and to an artificial sand of fused alumina. The attractive factor is present in water in which fresh Bullhill Bank sand had been shaken.

A similar transference of a repellent factor from fresh Salthouse Lake sand is not so clearly demonstrable. Water in which fresh Salthouse Lake sand has been shaken appears to contain the attractive factor. Thus both attractive and repellent factors may be present in Salthouse Lake sand.

Activated charcoal stimulates larvae to settle and metamorphose, but needs to be in granular form, or associated with neutral grains, to exert its full influence. The charcoal contains copper in minute proportion, but whether this has any significance for metamorphosis is undetermined.

The grade of a sand was again shown to be a settlement factor of some importance. The most attractive grain sizes appear to be those of which the bulk of the Bullhill Bank surface sand is composed.

Analysis shows that the surface sand of Salthouse Lake (St. II) contains some three times the organic nitrogen content of the surface sand of the Bullhill Bank.

TABLE I. GRADING OF SURFACE SANDS

Date	(6) >26 mesh	(5) 26–40 mesh	(4) 40–60 mesh	(3) 60–86 mesh	(2) 86–100 mesh	(1) 100–200 mesh	Silt	Mean category	Percentage floatability
				From the middle of Bullhill Bank					
10. vi. 52	0·2	1·1	25·0	61·2	11·7	0·7	Slight	(3·15)	20–30
8. vii. 52	0·2	0·9	20·2	63·7	13·8	1·1	Slight	(3·07)	20–30
				From the Salthouse Lake (St. II)					
10. vi. 52	0·3	1·1	16·0	33·9	32·0	16·7	Fair quantity	(2·54)	50–60

Categories (1) to (6) are percentage mesh sizes by weight, after washing away silt.

TABLE II. SCALE OF WORDS USED TO EXPRESS NUMBER IN TABLES III–XI

Very few (sometimes also expressed in numbers 1–4)
Few
Several
Fair number
Good number
Many
Very many
Multitude

TABLE III. EXPERIMENT 52

(Begun 16. vi. 52 with larvae from a fertilization of 11. vi. 52.)
Results on 18. vi. 52.

Series A

	Bullhill Bank sand (10. vi. 52)			Salthouse Lake sand (10. vi. 52)	
	Rinsed in sea water	Incubated in medium for 6 days	Incubated in medium for 6 days after inoculation with Salthouse Lake sand (10. vi. 52)	Rinsed in sea water	Incubated in medium for 6 days
Settlements in Dish					
Metd	Very many	None	None	Several	None
Meting		3 or 4	2 or 3		2
Unmet.	Few	Several	Several		Several
Settlements in Conical Vessels					
Metd	Multitude, mainly metd.	Many	Many	Fair number, mainly meting	Several
Meting					
Unmet.	None	Several	Several	Several	

Series B

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands *Settlement in Conical Vessel*
Settlements in Dish				
Metd	Multitude, mainly metd.	None	Many, mainly metd.	Multitude, almost all metd.
Meting		2		
Unmet.	Few	1	Few	None

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands *Settlement in Conical Vessel*
Settlements in Dish				
Metd	Multitude, mainly metd.	None	None	Very few
Meting		None	1	
Unmet.	Several	Several	Several	Few

TABLE IV. EXPERIMENT 53.

(Begun 17. vi. 52 with larvae from a fertilization of 12. vi. 52.)
Results on 19. vi. 52.

Series A

		Acid-cleaned Bullhill Bank sand (9. v. 52) In bolting-silk envelope in sea water for 5 days		
	Stored in distilled water	In clean dish	Amid Bullhill Bank sand (10. vi. 52)	Amid Salthouse Lake sand (10. vi. 52)
		Settlements in Dish		
Metd	1	Few	Many, mainly metd.	None
Meting	1			None
Unmet.	Few	Few	Few	Few
		Settlements in Conical Vessels		
Metd	Good number	Fair number	Multitude, mainly metd.	Few
Meting	Good number	Fair number		Few
Unmet.	Good number	Fair number	Few	Few

Series B

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) soaked 1 hr. in 5 % formalin in sea water	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	Multitude, mainly metd.	None	*c.* 5	Many, mainly metd.
Meting		None	2	
Unmet.	Few	None	1	None

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) washed (distilled water), boiled in distilled water 2–3 hr.	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	Multitude	None	Fair number, mainly metd.	Multitude
Meting	Few	None		Very few
Unmet.	1 or 2	None	Several	None

Series D

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, treated activated charcoal 2–3 hr., normal sterilization	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	Multitude	Fair number	Multitude	Multitude
Meting	Very few		Few	Very few
Unmet.		Fair number	Few	None

15-2

TABLE V. EXPERIMENT 54

(Begun 25. vi. 52 with larvae from a fertilization of 20. vi. 52.)
Results on 27. vi. 52.

Series A

	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Salthouse Lake sand (10. vi. 52) boiled in distilled water 2–3 hr.	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	2 or 3	None	None	None
Unmet.	None	None	None	None

Series B

	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Salthouse Lake sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	1 or 2	None	None	None
Unmet.	Few	3 or 4	3 or 4	Very few

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Oolitic sand, normal sterilization	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	Multitude	1	Many	} Multitude
Meting	Several	None	Several	
Unmet.	None	2	Few	None

Series D

	Salthouse Lake sand (10. vi. 52) washed, dried, normal sterilization	Salthouse Lake sand (10. vi. 52) washed, dried, activated charcoal ¾ hr., normal sterilization	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	None	Fair number	None	Very few
Unmet.	1 or 2	Fair number	Few	Many

TABLE VI. EXPERIMENT 55

(Begun 30. vi. 52 with larvae from a fertilization of 25. vi. 52. Results on 2. vii. 52.)

Series A

Acid-cleaned Salthouse Lake sand (10. vi. 52)

		In bolting-silk envelope in sea water for 6 days		
	Stored in distilled water	Amid acid-cleaned Bullhill Bank sand (9. v. 52)	Amid Bullhill Bank sand (23. vi. 52)	Amid Salthouse Lake sand (23. vi. 52)
		Settlements in Dish		
Metd	1 or 2	1 or 2	25–30	None
Meting	1 or 2	1 or 2	Few	2
Unmet.	Few	Several	Several	Few
		Settlements in Conical Vessels		
Metd	1 or 2	3 or 4	Very many	5 or 6
Meting	Few	Few	Several	Few
Unmet	Good number	Good number	Several	Several

Series B

	Bullhill Bank sand (23. vi. 52) rinsed in sea water	Bullhill Bank sand (23. vi. 52) kept in distilled water for 3 days	Mixture of the two sands
		Settlements in Dish	
Metd	Multitude	Good number	Many
Meting	Few	Few	Few
Unmet.	None	None	None
		Settlements in Conical Vessels	
Metd	—	Multitude	Multitude
Meting	—	Few	Few
Unmet.	—	None	None

Table VII. Experiment 56

(Begun 1. vii. 52 with larvae from a fertilization of 25. vi. 52. Results on 3. vii. 52.)

Series A

Acid-cleaned Bullhill Bank sand (10. vi. 52)

	(1) Stored in distilled water	(2) In sea water in light for 10 days	(3) Sand no. 2 to which a few grains of Bullhill Bank sand (23. vi. 52) were added on 1. vii. 52	(4) On 21. vi. 52 a few grains of Bullhill Bank sand (10. vi. 52) added. In sea water in light for 10 days	(5) Sand no. 2 to which a few grains of Salthouse Lake sand (23. vi. 52) were added on 1. vii. 52	(6) On 21. vi. 52 a few grains of Salthouse Lake sand (10. vi. 52) added. In sea water in light for 10 days
Settlements in Dish						
Metd	Few	Few	Several	Good number	Good number	Many
Meting	Several	Several	Few	Several	Several	Several
Unmet.	Several	Several	Few	Few	Few	Few
Settlements in Conical Vessels						
Metd	Several	Many	Many	Very many	Many	Multitude
Meting	Very many	Many	Many	Many	Many	Many
Unmet.	Many	Several	Several	Very few	Few	Very few

Series B

Acid-cleaned Bullhill Bank sand (10. vi. 52), treated exactly as for Series *A* but nos. 2 (including 3 and 5), 4 and 6 kept in the dark for 10 days

	(1)	(2)	(3)	(4)	(5)	(6)
Settlements in Dish						
Metd	Few	Few	Several	Many	Several	Many
Meting	Several	Few	Several	Several	Several	Several
Unmet.	Several	Few	Several	Few	Few	Few
Settlements in Conical Vessels						
Metd	*See A 1 above*	Many	Many	Very many	Many	Very many
Meting		Many	Many	Good number	Good number	Good number
Unmet.		Good number	Several	Few	Few	Very few

Series C

	Bullhill Bank sand (23. vi. 52) normal sterilization	Oolitic sand, normal sterilization	Mixture of the two sands
Settlements in Dish			
Metd	None	2	None
Meting	Few	Few	Very few
Unmet.	Few	Few	Few
Settlements in Conical Vessels			
Metd	Very many	Many	Many
Meting	Many	Many	Many
Unmet.	Very few	Good number	None

Series D

	Bullhill Bank sand (23. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, dried, normal sterilization	Mixture of the two sands
Settlements in Dish			
Metd	Multitude	None	None
Meting	Good number	None	2 or 3
Unmet.	None	3 or 4	3 or 4
Settlements in Conical Vessels			
Metd	—	Several	Many
Meting	—	Several	Many
Unmet.	—	Several	Many

TABLE VIII. EXPERIMENT 57

(Begun 14. vii. 52 with larvae from a fertilization of 9. vii. 52.)
Results on 16. vii. 52.

Series A

Oolitic sand from the Great Salt Lake

	(1) In bolting-silk envelope amid Oolitic sand in sea water for 16 days	(2) Sand no. 1 to which a few grains of Bullhill Bank sand from no. 3 were added on 14. vii. 52	(3) In bolting-silk envelope amid Bullhill Bank sand (23. vi. 52 and 8. vii. 52) in sea water for 16 days	(4) Sand no. 1 to which a few grains of Salthouse Lake sand from no. 5 were added on 14. vii. 52	(5) In bolting-silk envelope amid Salthouse Lake sand (23. vi. 52 and 8. vii. 52 in sea water for 16 days
Settlements in Dish					
Metd	3	2	11	1	None
Meting	3	5	8	1	None
Unmet.	Several	Several	Several	Several	Several
Settlements in Conical Vessels					
Metd	Good number	Good number	Good number	Good number	Few
Meting	Good number	Good number	Good number	Good number	Few
Unmet.	Several	Several	Several	Several	Good number

Series B

Acid-cleaned Bullhill Bank sand (10. vi. 52)

		Kept for 16 days, in the light in		
	Stored in distilled water	Filtered sea water	Sea water in which Bullhill Bank sand (23. vi. 52) had been shaken	Sea water in which Salthouse Lake sand (23. vi. 52) had been shaken
Settlements in Dish				
Metd	None	Few	Many	Several
Meting	1 or 2	Few	Good number	Several
Unmet.	Several	Few	Many	Several
Settlements in Conical Vessels				
Metd	Several	Good number	Very many	Many
Meting	Good number	Good number	Very many	Many
Unmet.	Many	Many	Many	Severa

Series C

Acid-cleaned Bullhill Bank sand (10. vi. 52)

	(1) Stored in distilled water	(2) Sand no. 1, to which on 14. vii. 52 1 %, or less, of Bullhill Bank sand (8. vii. 52) was added	(3) Sand no. 1 to which on 14. vii. 52 1 %, or less, of Salthouse Lake sand (8. vii. 52) was added
Settlements in Dish			
Metd	None	None	None
Meting	None	None	None
Unmet.	Good number	Good number	Good number
Settlements in Conical Vessels			
Metd	—	1 or 2	1 or 2
Meting	—	Few	Few
Unmet.	—	Good number	Several

TABLE IX. EXPERIMENT 58

(Begun 15. vii. 52 with larvae from a fertilization of 10. vii. 52.)
Results on 17. vii. 52.

Series A

Fused alumina

	Stored in distilled water	In bolting-silk envelope in sea water for 7 days		
		In clean dish	Amid Bullhill Bank sand (8. vii. 52)	Amid Salthouse Lake sand (8. vii. 52)
		Settlements in Dish		
Metd	2	1	26	1
Meting	2	4	32	None
Unmet.	Fair number	Fair number	Many	Fair number
		Settlements in Conical Vessels		
Metd	1 or 2	Several	Several	1 or 2
Meting	Several	Fair number	Many	Several
Unmet.	Many	Many	Very many	Several

Series B

	Acid-cleaned Bullhill Bank sand (10. vi. 52)			
	Stored in distilled water	Mixed with activated charcoal powder	Small grains of activated charcoal	Granular activated charcoal
		Settlements in Dish		
Metd	None	Many	Several	Very many
Meting	None	Many	Several	Very many
Unmet.	None	Several	Very many	Good number
		Settlements in Conical Vessels		
Metd	Few	Multitude	—	Very many
Meting	Several	Multitude	—	Very many
Unmet.	Very many	Few	—	Very many

TABLE X. EXPERIMENT 59

(Begun 16. vii. 1952 with larvae from fertilizations of 9. vii. 1952 and 10. vii. 1952.)
Results on 18. vii. 1952.

Acid-cleaned Bullhill Bank sand (10. vi. 1952).

	Stored in distilled water	Incubated in medium for 5 days	Inoculated with Bullhill Bank sand (8. vii. 1952) and incubated in medium for 5 days
		Settlements in Dish	
Metd	1 or 2	Fair number	Many
Meting	3 or 4	Fair number	Many
Unmet.	Few	Good number	Fair number
		Settlements in Conical Vessels	
Metd	Few	Few	Few
Meting	Fair number	Good number	Many
Unmet.	Many	Many	Many

TABLE XI. EXPERIMENT 60

(Begun 19. vii. 1952 with larvae from a fertilization of 14. vii. 1952.)
Results on 21. vii. 1952.

Bullhill Bank sand (8. vii. 1952) graded by sieving through bolting silks in filtered sea water

	Sand shaken on 100-mesh silk in sea water	Sand grains >26-mesh sizes	Sand grains 26–40-mesh sizes	Sand grains 40–60-mesh sizes	Sand grains 60–86-mesh sizes	Sand grains passing 86-mesh	Sand grains passing 100-mesh
				Settlements in Dish			
Metd	7	None	6	12	7	3	1
Meting	3	None	1	4	1	None	None
Unmet.	Few	Few	3	Few	Few	1 or 2	1 or 2

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THE BIOLOGY OF *ASTERIAS RUBENS* L.

V. A PORPHYRIN PIGMENT IN THE INTEGUMENT*

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(Text-figs. 1 and 2)

A porphyrin was extracted by MacMunn (1886), working at Plymouth, from the integument of *Asterias rubens* then known as *Uraster rubens*. He extracted whole starfishes with ethanol and ammonia and also with ethanol and sulphuric acid. After diluting his acid-ethanol solution with water he shook the extract with chloroform; the chloroform solution showed two absorption bands, at 607–593 and 566–548 mμ. On evaporation this extract yielded a brown amorphous residue which he found to be soluble in absolute ethanol giving a red solution with the same absorption spectrum. On adding ammonia four absorption bands appeared, at 632–622, 586–566, 548–529 and 516–490 mμ. He considered that this and similar pigments from the slug *Arion empiricorum*, and the coelenterates *Flabellum variabile* and *Fungia symmetrica* were identical with the haematoporphyrin of Hoppe-Seyler (1871) which was then the only porphyrin known. Experimental work has now shown that in other animals examined and found to contain porphyrin this pigment is never present in the form of haematoporphyrin. A reinvestigation has therefore been made of the occurrence of porphyrin in the starfish, *Asterias rubens*.

Certain details of the distribution in the Plymouth area of different colour forms of *A. rubens* have already been recorded (Vevers, 1949). In general, starfishes on the Rame-Eddystone grounds are dark brown or red-brown, but specimens coloured violet, violet-brown, red or even pale pink are not uncommon. In Plymouth Sound, on the other hand, a pure population of bright red starfishes was found in 1948; this population has now been greatly reduced in number but specimens can still be obtained.

In the present work four different colour types were used. The choice of these types was arbitrary, but they were nearly all colours which could be easily recognized in a sample of this species. The colour types used were dark brown, violet-brown and pale, all from the Rame-Eddystone grounds, and red (bright red) from the Plymouth Sound population. The violet-brown specimens were very close to the var. *violacea* of earlier writers.

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[*] A shortened form of this paper was read by one of us (G.Y.K.) at the 2nd International Congress of Biochemistry in Paris 1952.

Chemical study of the pigment was carried out in two parts: (*a*) isolation and identification of the porphyrin, and its preparation in quantity; (*b*) determination of the porphyrin contents per gram of integument in starfishes of different colour groups.

Isolation and Identification of the Porphyrin

Isolation of Crude Free Porphyrin

The aboral integument of *Asterias* was stripped off with scissors, separated carefully from the adherent digestive caeca and both integument and caeca were shaken separately with ether:glacial acetic acid (mixture 5:1) for 2 hr. (Zeile & Rau, 1937). The resulting extracts were washed with distilled water until most of the acetic acid was removed, and extracted with 5% HCl (137 ml. conc. HCl/l.). The acid layer from the integument extract was purple and red-fluorescent in ultra-violet light, and greenish brown and non-fluorescent from the caeca extract.

The red-fluorescent acid extract from the integument was treated with fresh ether and saturated sodium acetate solution added to drive the porphyrin back into the ether. The ether extract was then washed free of salts with distilled water, dried roughly by filtering through ether-soaked paper, and evaporated to dryness.

A violet-red residue remained, which was used for subsequent experiments.

Hartridge Reversion Spectroscope

A little of the pigment dissolved in 'Analar' pyridine gave a spectrum showing the following bands:

I	II	III	IV
632	576	542	507 mμ

corresponding with that of *protoporphyrin.*

Chloroform solutions of the methyl ester of this pigment and of protoporphyrin dimethyl ester (prepared from haemoglobin) gave the following bands:

	I	II	III	IV
Asterias porphyrin methyl ester	630	575	540	507 mμ
Protoporphyrin dimethyl ester	631	575	541	507 mμ

Unicam Spectrophotometer

An absorption spectrum of a solution of the pure pigment in pyridine ('Analar') was recorded with a Unicam S.P. 500 Quartz Spectrophotometer. This spectrum is plotted in Fig. 1, and shows absorption maxima at:

624 568 535 500–506 406 mμ

Acid Test of Chu (1946)

A little of the porphyrin was dissolved in 'Analar' chloroform and 3 drops conc. HCl added. The solution turned *green* suggesting *protoporphyrin*. (Mesoporphyrin gives a purple colour.)

Paper Partition Chromatography

A small amount of the free porphyrin was dissolved in lutidine (a mixture of 2:4- and 2:5-dimethyl pyridines) and partition chromatograms were run on strips of Whatman no. 1 paper 3·1 × 75 cm. at a temperature of 24° C. in an atmosphere of lutidine, water and ammonia vapour (Nicholas & Rimington, 1949) in a new apparatus for paper chromatography (Kennedy, 1953*b*). Pure protoporphyrin and coproporphyrin were used as markers, both in separate and in mixed spots. Papers were examined by ultra-violet light from an 'Osira' 125 W. black glass lamp after 15–16 hr. chromatography.

With protoporphyrin the *Asterias* porphyrin gave one spot only, and with coproporphyrin, two spots (Fig. 2).

A free *Asterias* porphyrin spotted on paper alone from lutidine solution gave one spot only, with an R_F value of 0·8, indicating the presence of a dicarboxylic porphyrin only, there being no other spots on the paper.

Column Chromatography

A sample of the porphyrin was esterified with methanol saturated with HCl gas at 0° C., and the dimethyl ester was dissolved in 'Analar' chloroform and applied to a column of grade III MgO (Nicholas, 1951) packed in chloroform. The chromatogram was developed by successive mixtures of chloroform: methanol in the proportions 100:0·5, 100:2 and 100:3 (Nicholas, 1951). Elution occurred only with the 100:3 chloroform:methanol mixture, indicating protoporphyrin and not mesoporphyrin or deuteroporphyrin.

Melting-point Determination

After chromatographing three times on grade III magnesium oxide the porphyrin dimethyl ester crystallized from chloroform and methanol in red prisms, melting at 225° C. A mixture of the porphyrin dimethyl ester with pure protoporphyrin dimethyl ester melted at 226° C.

Although Jope & O'Brien (1945) have criticized melting-point determinations as methods of identification of porphyrins, these results would indicate the identity of the *Asterias* porphyrin with protoporphyrin.

Formation of Lead Co-ordination Complexes

Lead co-ordination complexes were prepared from *Asterias* porphyrin, from pure protoporphyrin and from haematoporphyrin. All gave two-banded spectra:

	I	II
Pb—*Asterias* porphyrin	630	583
Pb—Protoporphyrin	630	584
Pb—Haematoporphyrin	620	578

This result indicates the identity of *Asterias* porphyrin with protoporphyrin and distinguishes it from haematoporphyrin.

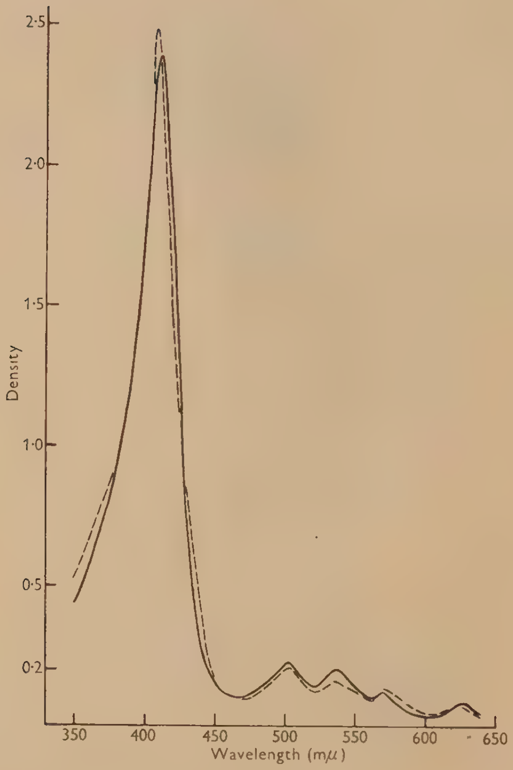


Fig. 1.

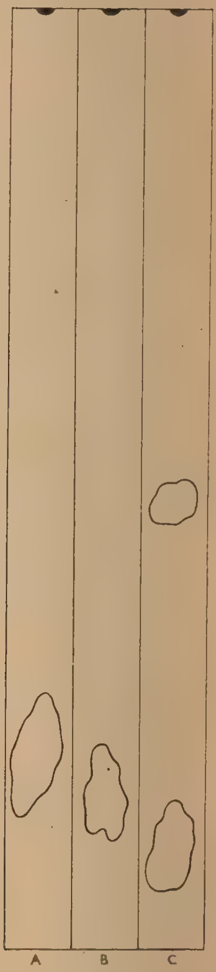


Fig. 2.

Fig. 1. Absorption spectra in pyridine of protoporphyrin (continuous line) and *Asterias* porphyrin (broken line).

Fig. 2. One-dimensional paper chromatograms run for 15–16 hr. at 24° C. in lutidine. The starting-points are marked as black semicircles: A, *Asterias* porphyrin alone; B, *Asterias* porphyrin and free protoporphyrin, mixed spot; C, *Asterias* porphyrin and coproporphyrin, mixed spot.

Conversion to Mesoporphyrin

The *Asterias* porphyrin was reduced according to the method of Rimington (1938). The solid porphyrin was dissolved in 20 ml. of a mixture of glacial acetic acid (7·5 parts) and hydriodic acid (sp.gr. 1·94) (1 part) and boiled for 3 min. After cooling and adding peroxide-free ether, the excess iodine was removed with sodium acetate and sodium sulphite. The ether epiphase gave a spectrum of mesoporphyrin

I	II	III	IV
623	567	528	495 mμ

The ether layer was evaporated and the porphyrin esterified with methanol/HCl at room temperature and extracted with chloroform. This solution was diluted with ether and the porphyrin was taken into 5% (w/v) HCl. The porphyrin was then driven back into the ether with potassium acetate and the ether layer washed, dried by filtering through an ether-soaked paper, and evaporated. The residue was crystallized from chloroform-methanol, and melted at 212–214° C. A mixed melting-point with a specimen of mesoporphyrin IX di-methyl ester (m.p. 215–216° C.) gave 215° C.

This reaction enabled the original *Asterias* porphyrin to be characterized as being of similar constitution to blood pigments derived from aetioporphyrin III, and it could only be protoporphyrin IX, the vinyl groups having been hydrogenated to ethyl groups in mesoporphyrin.

These experiments confirm that *Asterias rubens* integument contains protoporphyrin IX and that the porphyrin is present in the free state.

Chlorophylls in Caeca

Digestive caeca were dissected out of living *Asterias* and ground with acid-washed glass powder in a mortar, and then extracted with 90% methanol. A deep green solution with a marked rosy-red fluorescence was obtained. This solution gave the spectrum of a chlorophyll mixture and was further identified by the formation of various degradation products of chlorophyll. There was no evidence of the presence of a porphyrin in the digestive caeca.

PREPARATION OF *ASTERIAS* PORPHYRIN

The aboral integument was dissected from a number of large red-brown living *Asterias*. This was scraped free of adhering caeca, washed in sea water, blotted on filter-paper and weighed. The weight of integument as starting material was 548·5 g.

The pooled integument was put through a tissue mincer, and mechanically shaken with 2500 ml. of ether-acetic acid mixture for 5 hr.

The ether-acetic acid mixture was made up as follows: peroxide-free ether ('Analar'), 5 parts; glacial acetic acid ('Analar'), 1 part; then petroleum ether 40–60° C. m.p. ('Analar'), 1 part, added to 9 parts of above mixture.

The petroleum ether helps to avoid emulsification which can be troublesome.

All the following operations were done in very subdued light to minimize the decomposition of the porphyrin.

After shaking, the mixture was allowed to stand overnight in the ice-chest. The following day the mixture was filtered, and the residue re-extracted with a further 1500 ml. of the ether-acetic acid solvent. This was repeated with 1000 ml. lots until the extract was no longer red-fluorescent to ultra-violet light. Even then, the residue was found to be strongly fluorescent due to adsorption of porphyrin on the calcareous spicules of the integument. The residue was therefore shaken with 25% by weight HCl and re-extracted (after neutralization with saturated sodium acetate until Congo-red paper gave a *grey* colour) with 500 ml. lots of ether until the ether-layer was no longer red-fluorescent to ultra-violet light. All the extracts were pooled.

The extracts were first washed with distilled water containing a little sodium acetate, and then with distilled water alone until most of the acetic acid had been removed. The porphyrin was then extracted from the ether solution with 100 ml. portions of 5% by weight HCl until the extracts were no longer fluorescent.

The acid extracts were then pooled in a separating funnel and the porphyrin driven back into 'Analar' ether with saturated sodium acetate. The ether layers were pooled, and washed, first with 2% NaCl, followed by distilled water until washings were no longer acid, and until they no longer gave a precipitate with silver nitrate. During washing a little 'Analar' ether was added from time to time to replace that removed by washing. (Ether dissolves in water to the extent of 7·5% at 20° C.)

All drops of water were shaken down and tapped off, the ether extract filtered through thick paper soaked with ether, into a distilling flask, and reduced to a small volume by distillation *in vacuo*.

The residue was poured into a 500 ml. beaker and evaporated to dryness on the water bath. The porphyrin remaining was rinsed with a little petroleum ether (b.p. 40–60° C.) when cool, and dissolved in the minimum amount of 'Analar' pyridine. This solution was chilled, and dry petroleum ether b.p. 60–80° C. added in the proportion of about 10 volumes with stirring. The porphyrin was precipitated and flocculated by placing the beaker on the hot-water bath until large flocks developed. The beaker was then well covered and, placed in the ice-chest overnight.

The supernatant liquid (if free from flocks) was pipetted off, and the remainder centrifuged. The residue was then washed with petroleum ether (b.p. 40–60° C.) and dried *in vacuo* at room temperature.

The total yield of crude porphyrin by this method was 38·4 mg.

Purification

The dry porphyrin was dissolved in absolute methanol saturated at 0° C. with dry HCl gas, and the mixture allowed to stand in the ice-chest for 48 hr. This esterified the porphyrin.

The solution in methanol/HCl was diluted with a little distilled ice water, chloroform was added with shaking, and the lower layer was tapped off. The whole process was repeated until the lower layer was no longer red-fluorescent. The pooled chloroform layers were washed carefully with 2% NaCl, followed by distilled water, the washings being monitored with ultra-violet light. Washing was continued until the washings were neutral to litmus.

The chloroform extract was filtered through a thick chloroform-soaked paper into a distilling flask, and the extract concentrated to small bulk, then transferred to a porcelain basin and taken to dryness on the water bath.

The dry residue was rinsed, when cool, with petroleum ether (b.p. 40–60° C.) and dissolved in a mixture of chloroform-petroleum ether 1:1, and passed down a column of Brockmann's alumina, packed in chloroform-petroleum ether 1:1 and monitored with ultra-violet light. (A new apparatus for fractional elution chromatography was used, Kennedy, 1953*a*.)

The main portion of the pigment passed through the column, preceded by a blue fluorescent band which was found to be due to fat. At the top of the column, a dark brown pigment remained which was eluted later with chloroform/acetic acid, and gave a greenish fluorescence and two-banded very indistinct spectrum (Eriksen, 1951).

The main bulk of the porphyrin passing through the column was collected and evaporated to dryness in the water bath. The pigment was then dissolved in pure dry chloroform and applied to a column of magnesium oxide grade III packed in chloroform. Development was done by successive mixtures of 0·5:100, 2:100 and 3:100 (v/v) methanol-chloroform (Nicholas, 1951). Only the 3:100 mixture caused elution, indicating the presence of protoporphyrin only.

It may be helpful to include here some details of the crystallization method from chloroform-methanol, a method which often leads to disappointment.

The eluted porphyrin was evaporated to dryness in a porcelain basin on the water bath, and redissolved, on the water bath, in a small volume of dry 'Analar' chloroform. This was allowed to evaporate *slowly* until the effect of 'tears of wine' was apparent on the sides of the basin: i.e. the solvent running back in streaks from a band about 5 mm. above the surface. At this point, 3 volumes—estimated roughly by eye—of *boiling* dry absolute methanol were added with stirring, and crystallization began at once. The basin was covered and set aside in the ice-chest for further crystallization overnight.

The crystals were removed by centrifugation, keeping the temperature as low as possible, and drained by standing the tube on end on a pad of filter-paper. They were then washed with dry petroleum ether (b.p. 40–60° C.).

The mother liquor was evaporated to dryness and the crystallization process repeated.

After two recrystallizations of the mother liquors, the crystals were dried *in vacuo* over P_2O_5 at room temperature. A yield of 33·6 mg. of fine dark red prisms was obtained, melting at 225° C.

It is essential that all solvents should be pure and dry. Methanol was dried by the Mg and I_2 method of Vogel (1948). Chloroform was dried over anhydrous calcium chloride for 72 hr. and then redistilled with a calcium chloride tube attached to the receiver. Petroleum ether, though 'Analar', was dried over calcium chloride and sodium chippings and redistilled.

DETERMINATION OF PROTOPORPHYRIN IN *ASTERIAS* INTEGUMENT

There are difficulties in the determination of protoporphyrin in the integument of *Asterias*, of which the following may be mentioned. (i) The presence of calcium spicules in the integument and consequently the presence of calcium salts during the extraction process is a nuisance when adjusting the pH with sodium acetate. (ii) Protoporphyrin in small concentrations is unstable to light, and the estimation process must be done rapidly in dull light. (iii) Since protoporphyrin has a high acid number, especially in the form of the dimethyl ester, the use of 8% HCl is required in the preparation of the final extracts, and this gives a fluorescence which is less than maximal according to Rimington (1943).

However, it was considered that even an approximate determination of the amount of porphyrin in the various colours of *Asterias* would make a desirable and interesting comparison, and a modified technique was devised which took all the difficulties into consideration and gave reproducible results, using the Rimington fluorimeter (Rimington, 1943).

The fluorimeter was calibrated using dilutions of a freshly prepared standard solution of protoporphyrin made up as described below. The lability of protoporphyrin necessitated fresh standard solutions for each determination.

Standard Solution of Protoporphyrin

1 mg. pure protoporphyrin IX was dissolved in 5 ml. 'Analar' glacial acetic acid and the volume was made up to 100 ml. with 8% HCl (219·2 ml. conc. HCl/l.). This solution contained 10 μg./ml. and was kept in a refrigerator in a black bottle. Dilutions for calibration of the Rimington fluorimeter (for these determinations) were made freshly as required from the solution, with 8% HCl in dim light, but the standard stock solution was not kept for longer than 2 days. Hence, although fluorescence in 8% HCl is not maximal, both standard and unknown solutions in estimations are exactly comparable.

Determinations on each colour group were done separately, each compared with a freshly made up standard of 2 μg./ml. protoporphyrin in 8% HCl.

Method of Determination

All steps were carried out in very subdued light.

(i) 2 g. aboral integument of each colour were weighed out separately, after drying roughly in a standard manner on Whatman No. 1 paper, into wide-mouthed stoppered bottles.

(ii) 100 ml. methanol saturated with HCl gas were added and the whole was stored at 0° C. for 36 hr. in the dark.

(iii) The extracts were filtered into separating funnels and the bottles, residues and papers washed with small volumes of 30 ml. methanol/HCl; the washings being added to the main extracts.

(iv) The extracts were diluted with 1 volume of distilled water and the carotenoids removed by shaking with petroleum ether (b.p. 40°–60° C.) until the *upper* layer was non-fluorescent to ultra-violet light. The upper layers were then discarded.

(v) Peroxide-free ether was then added to the lower layers, followed by saturated sodium acetate (red to congo red), and well shaken. This was repeated until the upper (ether) layers were non-fluorescent to ultra-violet light. The lower layers were discarded.

(vi) The ether extracts were pooled and washed with ice-cold distilled water repeatedly until the washings gave no cloudiness with silver nitrate. All drops were shaken down and tapped off.

(vii) The ether solutions were extracted repeatedly with 2 ml. lots of 8% (w/v) HCl until no further fluorescence was seen in the lower (acid) layer. The lower layers were collected in stoppered measuring cylinders, and the total volume noted. All drops in the funnels were shaken down, and the measuring cylinders were well shaken.

(viii) 5 ml. samples of the acid extract in Eggertz tubes were compared with a 2 μgm./ml. protoporphyrin standard in the Rimington fluorimeter. If the solutions were too strong they were diluted with 8% HCl and the dilution factor noted.

The means of five to ten readings were taken, and the porphyrin values read off from the calibration curve. The results are expressed as μgm. protoporphyrin/g. of integument.

Results

The starfishes were graded by colour as follows: violet-brown, VB; dark brown, DB; red, R; pale, P.

The results of the porphyrin estimations are set out in Table I. Each figure is the mean of three determinations on an extract derived from two or three selected starfishes of the appropriate colour.

TABLE I. PORPHYRIN IN *ASTERIAS*

Given in μgm./g.

	Grade of colour			
Determinations at	VB	DB	R	P
Plymouth	72·0	33·6	24·5	5·5
Sheffield	64·4	49·9	13·0	4·5
Sheffield	70·3	45·2	11·4	—
Sheffield	69·7	47·2	15·2	—

TESTS ON THE SOFT PARTS OF *CHLAMYS* AND *MYTILUS*

With a view to tracing the origin of the porphyrin in *Asterias rubens*, the soft parts of *Chlamys* and *Mytilus* were ground with washed sand and extracted with ether-acetic acid mixture. The extract was very faintly blue-fluorescent, but had no red-fluorescence at all. On standing exposed to light in the presence of hydrogen peroxide or quinhydrone no red-fluorescence developed, confirming the absence of a porphyrinogen.

DISCUSSION

The results confirm the earlier evidence of MacMunn that a porphyrin is, in fact, present in the starfish, *Asterias rubens*, but extend his observations to the identification of the pigment as protoporphyrin, and not haematoporphyrin.

The determinations of protoporphyrin given in Table I show that in *A. rubens* integument the amounts per gram of this pigment are graded approximately according to the colour of the starfish. In general the darker starfishes have more protoporphyrin than the lighter ones. The main visible colour of the dark-brown and violet-brown starfishes is not, however, entirely due to the presence of this porphyrin, for these colours are known to be mainly carotenoid (including carotiproteins) (Vevers, 1952). The dark brown or black reticulate pattern has been shown to be a melanin pigment (Vevers, in preparation). At the same time there appears to be some parallelism between the porphyrin content and the colour of the carotenoid pigmentation. The measurements are relative and not absolute; and although there is much variation in pigmentation in *A. rubens*, the range of figures in Table I gives an excellent indication of the order of the porphyrin content in these varying shades of starfish.

The chromatographic results indicate that the protoporphyrin is present in the starfish body in the free state and not as an ester, since it takes up the clear position of protoporphyrin on the paper and does not travel all the way with the mobile phase as do the esters.

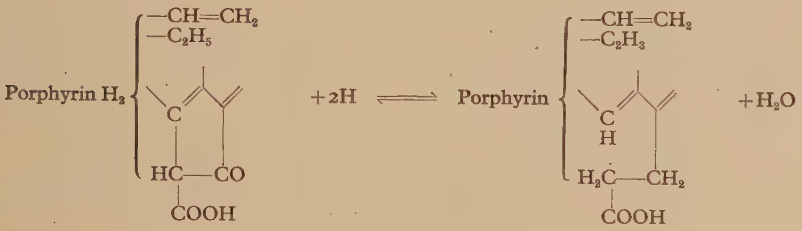
The problem of the origin of the free protoporphyrin in *Asterias* is intriguing. It is entirely absent from the soft parts of the lamellibranchs *Chlamys opercularis* and *Mytilus edulis*, which form the principal food of *Asterias rubens* in the Plymouth area. Uroporphyrin and coproporphyrin are widespread in mollusc shells, but do not occur in the soft parts that alone are eaten by the starfish.

The experiments with the soft parts of *Chlamys* and *Mytilus* have also shown that there is no extractable substance present comparable with a porphyrinogen, which could be converted simply to a porphyrin by oxidation in the starfish. The formation of protoporphyrin in *Asterias* must therefore be more metabolic.

There appear to be three possible sources of the free protoporphyrin: (i) haem enzymes and cytochromes, (ii) chlorophyll of the digestive caeca, (iii) complete synthesis *de novo* from simple substances in the diet.

Haem enzymes and cytochromes are present in the starfish in very small (physiological) amounts, and the amount of protoporphyrin in the integument is vastly greater. No transformation of the porphyrin *c* of cytochrome *c* (haematoporphyrin) to protoporphyrin has yet been demonstrated conclusively.

Chlorophyll is present in the digestive caeca in very large amounts, and could be converted into protoporphyrin by a dismutation, two hydrogen atoms being lost from the nucleus and two from the ethyl side chain. The addition of six hydrogen atoms would bring about opening of the isocyclic ring in chlorophyll and the formation of the second propionic acid side chain of protoporphyrin.



(*After Lemberg & Legge*, 1949.)

The third idea involves complete synthesis of the porphyrin nucleus from glycine, acetate, or α-ketoglutarate as shown by Muir & Neuberger (1950) and Shemin & Rittenberg (1946).

On the whole, the formation from chlorophyll is the most likely source of protoporphyrin in this organism, and will be the subject of future study.

The porphyrin in the integument clearly does not contribute to the colour pattern of the animal, as has been mentioned already. The pigment may therefore be either an excretory by-product formed from chlorophyll, or be concerned in integumentary photoreception, since porphyrins are known to render tissues more sensitive to light.

In the starfish the pigment apparently is only accumulated in the hard leathery integument, as repeated tests have shown it to be entirely absent from the gut, gonads and digestive caeca. Direct ultra-violet examination has also shown that there is no porphyrin pigment in the calcareous spicules embedded in the starfish integument. This is interesting since porphyrin is so frequently associated with calcium compounds, e.g. protoporphyrin in the hen's egg-shell, the porphyrins of mollusc shells (Nicholas & Comfort, 1949), and uroporphyrin in the bones of the squirrel *Sciurus niger*.

SUMMARY

A porphyrin pigment found in *Asterias rubens* has been re-examined and found to be protoporphyrin, and not haematoporphyrin as originally reported by MacMunn. The pigment was identified by spectroscopic and spectrophotometric observations as well as by chemical tests and the use of paper partition and column chromatography. The protoporphyrin is absent from the gut, gonads and digestive caeca, and is present only in the leathery integument.

The porphyrin occurs in the integument as free protoporphyrin; a yield of 33·6 mg. of red crystalline protoporphyrin dimethyl ester was obtained from a sample of 548·5 g. of *Asterias* integument.

The relative amounts of protoporphyrin in different coloured specimens of *A. rubens* were determined by a fluorimetric method. With four well-defined colour types the amount of porphyrin per gram of integument was found to decrease rapidly in the series: violet-brown, dark brown, red and pale.

Neither protoporphyrin nor a porphyrinogen was found in the soft parts of *Chlamys opercularis* or *Mytilus edulis*, the main food animals of *Asterias* in the Plymouth area. The exact source of the integumentary porphyrin in this starfish is therefore uncertain, but it is suggested that it may be derived from the chlorophyll pigments found in the digestive caeca.

The porphyrin does not contribute materially to the colour pattern of the animals, most of whose colour is carotenoid, and it is most likely that it is a by-product of excretion formed from chlorophyll.

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THE REGENERATION OF THE SIPHONS OF *CIONA INTESTINALIS* L.

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(Text-figs. 1–16)

The urochordates exhibit remarkable powers of asexual reproduction and regeneration, and have provided a fertile field for experiment along these lines. Much of this work has been performed upon ascidians such as *Diazona violacea* Savigny, *Archiascidia neapolitana* Julin, *Archidistoma aggregatum* Garstang and members of the Clavelinidae, etc., since they are relatively easy to obtain and their methods of budding are such as to lend themselves to experimental techniques. Species such as *Ciona intestinalis* (L.) and *Ascidiella aspersa* (Müller), etc., that do not reproduce asexually have, however, been little studied. A few experiments upon *Ciona intestinalis* have indicated that regenerative powers indeed exist, for they have shown that regrowth of the tunic, mantle, siphons, neuroglandular complex and much of the pharynx is possible. With the exception, however, of the regeneration of the tunic and neuroglandular complex, no detailed histological study of the processes involved has been undertaken. The present work attempts to remedy that deficiency with respect to the siphons and anterior end of the pharynx.

HISTORICAL

Hirschler (1914), using young *C. intestinalis*, discovered that the posterior region of the body could regenerate all the missing anterior organs (provided that a small portion of the pharynx were present). The anterior region could not, however, regenerate posterior organs. He noted that brown pigmented cells (the cells with orange pigment of Pérès, 1943) tended to accumulate at cut surfaces and attributed to them a phagocytic function. The numbers of regenerating 'ocelli' often varied considerably from those normal to the siphons.

Kammerer (1923) again demonstrated the ability of the siphons to regenerate, and added that the new growths were larger and longer than normal. He even stated that the offspring of the operated animals inherited the ability to grow longer siphons. This was disproved by Fox (1924) who showed that the length of the regenerating siphons was dependent upon nutrition and non-heritable.

Pérès (1948*a*) removed the tunic of *Ciona* and gave a very detailed account of the histology and chemistry of the growth of the new test. The epidermis and three types of cell, said by him to be mesenchymatous, are involved: (i) cells with granules and acidophil 'bâtonnets' at first carrying carbohydrates, later to be replaced by proteins; (ii) cells with a single, large, refringent granule of a protein nature; and (iii) cells with a small acidophil vacuole containing a carbohydrate of high molecular weight, possibly glycogen. The epidermis secretes the ground substance of the tunic, formed as a result of the polymerization of carbohydrates. This is speedily invaded by the three types of cells just described and the chemical changes necessary for the full development of the tunic are then completed.

The regeneration of the neuroglandular complex of *C. intestinalis* has been said by Pérès (1943) to be the work of 'neoblasts'. The nature of these cells will be discussed later.

Much of the work on regeneration and asexual reproduction in ascidians has been performed on *Clavelina lepadiformis* (Müller), by Huxley (1926), Brien (1930) and Berrill & Cohen (1936). Brien has shown that, while the main sources of newly differentiating tissues and organs in the bud chambers are mesenchymatous and blood cells which he calls lymphoblasts, in the artificial regeneration of the thorax in adults the epicardium is largely responsible. Berrill & Cohen, who isolated pieces of stolon artificially, state that the cells forming the blastogenic mass are derived from a mesenchymatous septum which has disintegrated. They appear not to recognize the participation of the blood cells, noted by Brien.

The work of Brien (1927) on *Aplidium zostericola* Giard. indicates that the majority of the new tissues, cardiopericardiac vesicle, pharynx, peribranchial cavities, epicardium and oesophagus, of the blastozooid are constituted from the fragment of epicardium in the bud. This appears also to be true of *Diazona violacea* Savigny, and *Archidistoma aggregatum* Garstang (see Berrill, 1948 *a*, *b*), and of *Archiascidia neapolitana* Julin (see Brien, 1933).

Selys Longchamps (1916) has described the method of pallial budding in *Heterocarpa glomerata* Hartmeyer, and *Stolonica socialis* Hartmeyer, and Drach (1948) that of the Botryllidae. In the stolidobranchs it is apparently ectoderm and mesenchyme which are responsible for organogenesis and tissue formation.

Berrill (1951), in a review of regeneration and budding in tunicates, states that in ascidians 'while the external tunic of cellulose is readily regenerated by exposed or renewed epidermis...regeneration of other tissues and structures may be accomplished by a variety of internal tissues depending upon the level of the zooid from which regeneration takes place....Siphons reform from epidermis and atrial epithelium....At post abdominal levels all structures other than epidermis are reconstituted from epicardial tissue alone when it is present, or from septal mesenchyme.'

Finally, Berrill (1950) is of the opinion that the epicardium is probably mesodermal and not endodermal as has hitherto been thought, but does not pursue the subject.

METHODS

The *Ciona intestinalis* upon which operations for siphon removal were performed were adults varying in length from 1·9 to 7 cm., averaging 3·7 cm.

The specimens were first anaesthetized in menthol in sea water for 1 hr. to avoid siphon retraction and then put into fresh sea water from Plymouth Sound. The water was changed daily and kept at a temperature varying between 15 and 16° C. The siphons were removed in such a way that the cut edges were flush with the body surface (Fig. 1). In several specimens this involved the removal of a small part of the anterior end of the pharynx.

Thirty-nine specimens were used for observations upon the living animal during the period of regeneration. In nine of these, both siphons were removed, in sixteen the inhalant, and in fourteen the exhalant siphons alone. In these experiments the animals were kept alive for a minimum of 6 days to cover the period of growth of siphons normal in all respects other than length. Two animals in which both mouth and atriopore were removed were kept alive for a fortnight for study of the further development of the newly regenerated organs. In five of the above operations the neuroglandular complex was also removed. In three further experiments, in which both siphons were removed the neuroglandular complex was damaged but not excised.

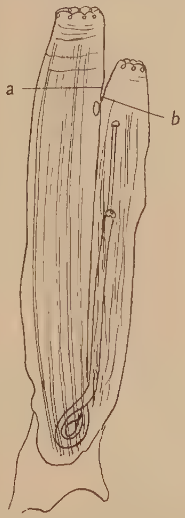

Fig. 1. *Ciona intestinalis*, showing level of cut for removal of inhalant siphon (*a*) and exhalant siphon (*b*).

Ninety-nine animals were operated upon for the removal of both siphons and then preserved for observations upon the histological changes involved in regeneration. The animals were killed at regular intervals, hourly up to 6 hr., two-hourly from 6 to 24 hr., and thereafter at four-hourly periods up to 96 hr., three being killed at each stage. The anterior thirds of the animals were fixed, either in Bouin-Dubosq or Benoit. The sectioned material was cut at 4 or 6μ and stained in Unna's pappenheim, or toluidine blue and eosin after Bouin-Dubosq, or with iron haematoxylin counterstained with light green after Benoit. The Feulgen test was carried out in many cases.

In addition, sixteen animals from which both siphons had been removed were used for experiments in *intra-vitam* staining. The material was immersed either in neutral red or janus green B. The concentration for each was 1 ml. of a 0·1 % solution of the stain in 9 ml. of sea water. Neutral red was used as an indicator of the presence of intracellular neutral red vacuoles, regarded by Baker (1945, 1949) as a component of the Golgi apparatus, janus green B as an indication of the distribution of mitochondria. Six injured and five uninjured animals were immersed in neutral red, and two injured and two uninjured in janus green B for a period of 24 hr. in each case.

Observations on the Living Material

For half an hour to 1 hr. after operation the effect of the anaesthetic is still evident; the reduced siphons, if the two apertures remaining after operation can be so described, still gape. From 1 to 2½ hr. later, however, the 'siphons' close, not by intrinsic muscular movements, since the sphincters have been removed, but by differential movements of the anterior end of the body. When one siphon alone has been removed, the anterior end bends over towards the operated side, thus effectively closing the aperture; when both have been removed, differential contractions of the mantle musculature result in the retraction of the siphon region into the anterior end of pharynx and atrium. However, in 3 hr. time the apertures are again open. Momentary stimulation with a dissecting needle fails to produce any closure of the actual apertures, and only prolonged stimulation provokes a repetition of the bending or retraction movements described above.

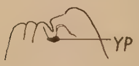

Fig. 2.

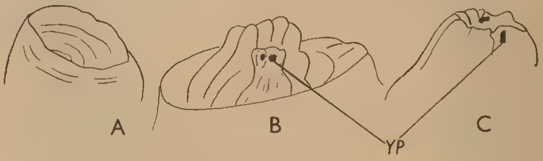

Fig. 3.

Fig. 2. Mouth, 1 day after operation. *YP*, yellow pigment.

Fig. 3. Two days after operation. A, mouth (specimen IV); B, mouth (specimen I); C, atriophore (specimen II).

During the first day (Fig. 2) certain reactions to the operation are observed. The cut edges bend inwards, and in 60% of the specimens are the site of the irregular deposition of yellow pigment, a phenomenon noted by Hirschler (1914).

In the course of the second day (Fig. 3) very slight growth of the siphons occurs, sufficient to restore a regular, but not characteristically lobed shape. The new growth is extremely delicate and there is no visible evidence of regeneration of a new tunic by the new tissue. In a few animals the yellow pigmentation becomes more intense, but in most it was noticeably paler, and from one had entirely disappeared.

The changes initiated during the second day continue into the third. As a result of continued but differential growth, the siphons acquire the characteristic lobes. Further, during this period, secretion of tunicin by the new tissues begins. The siphons now open and close normally, an indication of the presence of sphincter muscles. By the end of the fourth day the 'ocelli' have differentiated (Fig. 4).

Thus, in 4 days, siphons complete in all respects other than length are regenerated. There were only two exceptions. In one specimen the pigment spots appeared on the third day, and in another they were not visible until the fifth. The removal of only one instead of both siphons does not expedite the repair process for the stages and speed of regeneration are identical in each.

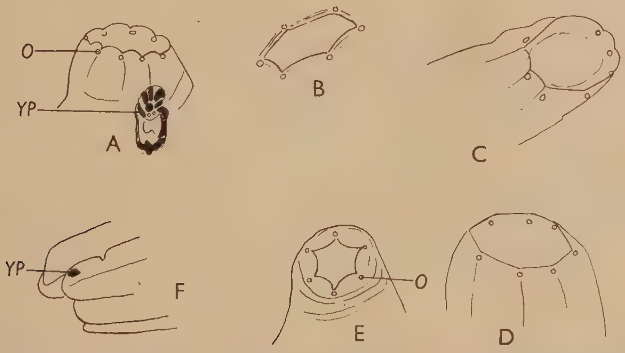

Fig. 4. Four days after operation. A, mouth (specimen I); B, atriophore (specimen II); C, mouth (specimen III); D, mouth (specimen IV); E, exhalant siphon: neuroglandular complex injured but left *in situ*; F, exhalant siphon: neuroglandular complex removed. *O*, ocellus (pigment spot); *YP*, yellow pigment.

However, a profound effect is produced by the removal of the neuro-glandular complex together with the siphons (Fig. 5). Regeneration of the siphons is much slower, not being completed until 13 or 14 days have elapsed. If the complex is damaged by maceration with a needle, but is not excised, the regeneration rate is normal.

The process of regeneration subsequent to the fourth day involves a thickening of the new tunic and continued growth in length of the siphons, the normal size being regained in 2–3 weeks after operation.

Neutral red staining was used to indicate the presence of intracellular 'neutral red vacuoles' regarded by Hirsch (1939) as the Golgi pre-substance, and by Baker (1945, 1949) as part of the Golgi apparatus. This cell organelle is generally regarded as being connected with secretory activity. It was therefore hoped that this technique would give evidence of increased secretory activity by the cells of the injured region. Observations over a period of 2 days in fact indicated that the injured region took up neutral red to a greater extent than the undamaged tissues. Microscopic examination after 24 hr. showed, however, that cells other than those with yellow pigment granules, muscle and test cells, betrayed a much greater affinity for the stain that had been expected.

Both cytoplasm and nucleus of haemoblasts, of cells with many refringent granules or with a single granule, of lymphocytes, and of amoebocytes took up the stain. The granules where present coloured more intensely than the cytoplasm. In phagocytes and in senescent cells in the tunic, the contents of the vacuoles took up the stain intensely; the cytoplasm and nucleus were coloured to a much smaller degree. As the senescent cells moved towards the edge of the tunic the intensity of the neutral red within the growing vacuoles steadily increased.

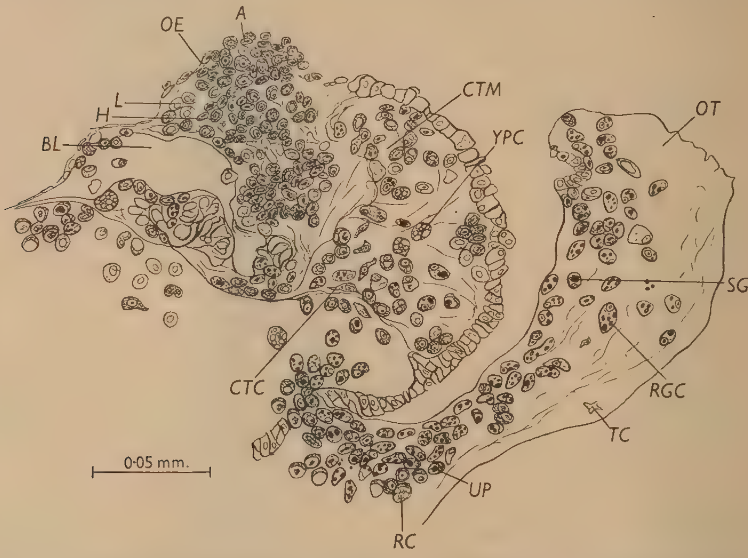

Fig. 5. Six hours after operation—atriopore. *A*, amoebocyte; *BL*, blood lacuna; *CTC*, connective tissue cell; *CTM*, connective tissue matrix; *H*, haemoblast; *L*, lymphocyte; *OE*, original epidermis; *OT*, original tunic; *RC*, reticulate cell; *RGC*, refringent granule cell; *SGC*, cell with single granule; *TC*, tunic cell; *UP*, univacuolar phagocyte; *YPC*, yellow pigment cell.

Janus green B was used in an attempt to stain mitochondria. The reactions observed were interesting, since, with minor exceptions, those regions within the cells which showed an affinity for neutral red also took up janus green B. So widespread was the staining within the cells that it was impossible, in general, to distinguish the mitochondria, which according to Pérès (1943) are never large.

OBSERVATIONS ON PRESERVED MATERIAL

Examination of sectioned material shows that during the first hour after siphon removal no visible changes in the affected tissues occur.

During the first 6 hr. after operation two reactions to the injury by the affected tissues can be distinguished. The first may be described as comprising direct traumatic responses to the operation; the second as comprising processes that represent the earliest stages in the regeneration of the new siphons.

The earliest direct traumatic response is the approximation and apposition of the cut epidermal edges of the mantle which bend inwards and meet (Fig. 6). At first they may overlap, but later they appear to fuse. The epidermal cells in this region alter in shape from characteristic small flattened cells to a cubical, and in places almost columnar, epithelium. In many specimens, where the two epidermal layers do not meet, the gap is plugged by a clump of orange pigment cells, the largest of the blood cells. In other regions the gap is plugged by muscle cells. The differing degrees of intensity of the yellow pigmentation round the cut siphon edges is thus explained. The effect of these processes is to protect the subepidermal tissue from exposure to the water.

The orange pigment cells appear not to have a very long life, since even at this stage a few can be seen degenerating, a process which can occur in two ways. The first is characterized by a change in colour of the pigmentation from yellow or orange to green, and finally by a complete disintegration of the cell and release of the granules into the neighbouring subepidermal regions. Very probably the freed granules are then removed by two types of blood cell, univacuolar phagocytes and amoebocytes. The second method is that described by Pérès (1943). The orange pigment cells again change in colour to green and become reticulate cells (Fig. 5) with an achromatic nucleus. In the hyaline compartments between the refringent bars, one or two small granules can be seen, but in time even these disappear.

Another direct result of the operation is the damage or destruction of cells at the site of the injury. The removal of these cells is performed by amoebocytes and univacuolar phagocytes (Fig. 5). These appear in large numbers from two sources: (i) by emigration from the blood stream, and (ii) by differentiation on the spot, from haemoblasts and lymphocytes. At this stage the number of haemoblasts, although increasing, is not yet large, and, while differentiation of the two types of phagocytic cells is to be observed, the number produced by this means can only be relatively small.

The mobilization of the cells that take part in the regeneration of the siphons begins during this period. These cells are the haemoblasts, the basic blood cells which originate on the epicardium. Examination of sections shows that the initial increase in numbers of the haemoblasts is only, in very small measure, due to the divisions of haemoblasts already present in the injured area (Figs. 5, 6). The number of dividing cells is always small, although it increases slightly during the period of regeneration. Examination of both injured and uninjured animals, vitally stained with neutral red, which facilitates the recognition of haemoblasts in living material, shows that the cavities of the tentacles form reservoirs of haemoblasts. From the bases of the tentacles of injured animals chains of these cells extend towards the cut edges of the siphons. There is no evidence of a similar migration in uninjured animals. Further examinations of portions of the epicardium from both injured and uninjured animals give pertinent results. While the epicardium of uninjured animals shows a large number of haemoblasts, that of injured animals shows a vastly increased population. So dense is it over considerable areas that the cells are actually touching each other.

Thus the operation would seem to stimulate the liberation of haemoblasts from the tentacles, and to increase the rate of proliferation of these cells by the epicardium. They then enter by way of the blood stream into the zone of regeneration.

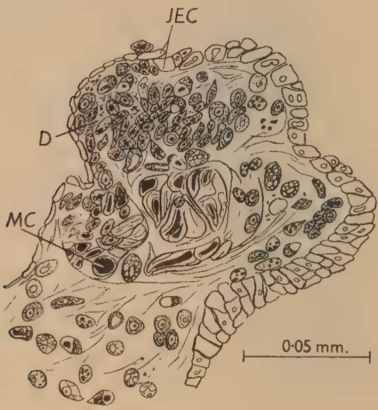

Fig. 6.

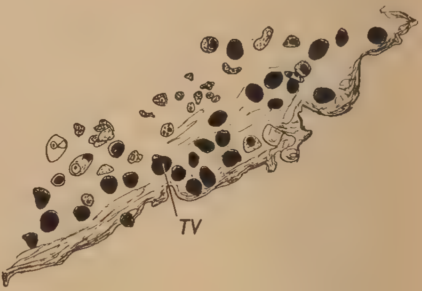

Fig. 7.

Fig. 6. Six hours after operation—mouth. The scale refers to this figure. *D*, debris; *JEC*, epidermal junction; *MC*, muscle cell showing extent of thymonucleic acid in cell.

Fig. 7. Edge of old test showing senescent cells. *TV*, cell with thymonucleic acid vacuole.

The first signs of differentiation from haemoblasts of the three types of cells that participate in the regeneration of the tunic can be distinguished 6 hr. after operation (Fig. 6). In addition, the number of these cells in a fully developed condition in the region has also increased. Some of these have already passed into the tunic adjacent to the old mantle (Fig. 6).

Finally, certain cells are eliminated from the animal by a process analogous to desquamation. Senescent cells, having migrated into the tunic, move towards the free edge and undergo degenerative changes *en route*. No matter what their origin, they all acquire a relatively enormous vacuole, and at the same time cytoplasm and nucleus decrease in volume. Ultimately only a crescentic area of cytoplasm containing a very small achromatic nucleus is to be distinguished (Fig. 7). The vacuole stains deeply with toluidine blue, the methyl green of Unna's Pappenheim, and is positive to Feulgen. These reactions suggest that as an accompaniment to the deterioration of the cells there is a nuclear breakdown which results in a liberation of thymonucleic acid into the vacuole. In other words, there appears to be a modified form of chromatolysis (cf. Wigglesworth, 1942). At the free edge the cells seem to drift together (Fig. 7) and as they pass out of the tunic become surrounded by a thin bag formed by the test. This finally breaks away from the animal altogether.

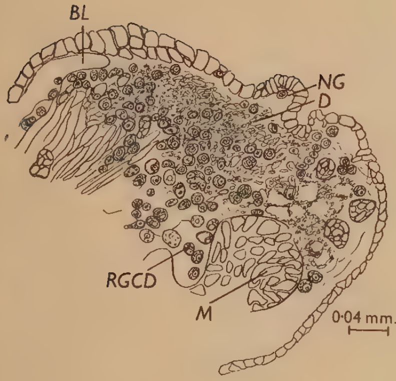

Fig. 8.

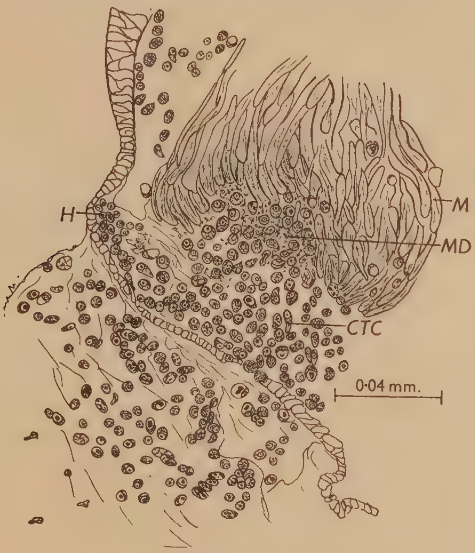

Fig. 9.

Fig. 8. Twelve hours after operation—mouth. *BL*, blood lacuna; *D*, debris; *M*, muscle; *NG*, new growth; *RGCD*, differentiating refringent granule cell.

Fig. 9. Sixteen hours after operation—mouth. *CTC*, connective tissue cell; *H*, haemoblast; *M*, muscle; *MD*, muscle debris.

Twelve hours after the operation (Fig. 8) the two epidermal layers have completely fused all round the reduced 'siphons'. In the subepidermal connective tissue there is a slight decline in the number of orange pigment cells, univacuolar phagocytes, amoebocytes and reticulate cells and an increase in the free pigment granules and in the numbers of small and medium-sized cells with refringent granules. The processes described as occurring 6 hr. after the operation continue.

By 16 hr. (Fig. 9) the numbers of those cells concerned with the regeneration of the missing siphons has markedly increased. The subepidermal lacunae are packed with haemoblasts and with cell-types differentiating from them. Univacuolar phagocytes, amoebocytes and yellow pigment cells remain, but they are now few.

In certain regions (Fig. 9) there appears to have been a mass migration of cells with one or several refringent granules into the tunic. In one specimen in which, as a result of the operation, the old test was torn from the epidermis posterior to the actual cut, new test growth was well advanced. The epidermal cells had already laid down the ground substance of the tunic, and cells with one or many granules were in the process of adding to the ground substance exactly as described by Pérès (1948*a*).

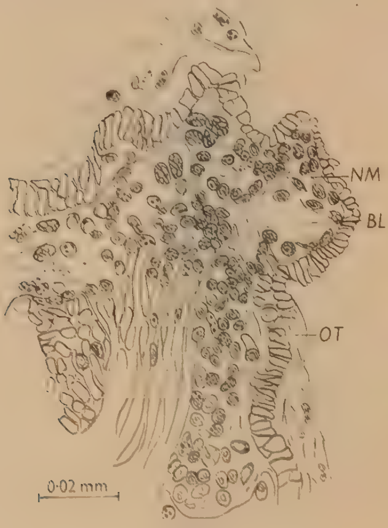

Fig. 10.

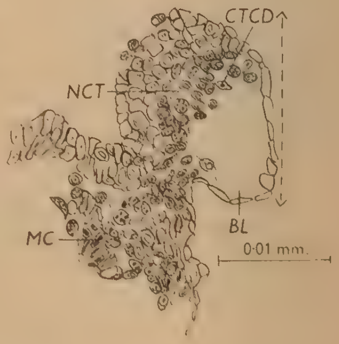

Fig. 11.

Fig. 10. Twenty-four hours—mouth. *BL*, blood lacuna; *NM*, new connective tissue matrix; *OT*, original tunic.

Fig. 11. Thirty-six hours—mouth. *BL*, blood lacuna; *CTCD*, differentiating connective tissue cell; *MC*, muscle cell; *NCT*, new connective tissue matrix; - - - , new growth.

In 24 hr. (Fig. 10) the haemoblast population is at its maximum density and the first evidence of actual regeneration of the mantle is observed. Between 16 and 24 hr. after the operation, the epidermal cells have begun to divide and a slight outpushing of the epidermis all round the cut edges of the siphons is apparent (Fig. 10). The bulge becomes filled with blood plasma carrying haemoblasts. Elsewhere in the region differentiation of haemoblasts and lymphocytes continues, in particular into cells with many granules or with a single granule, some of which migrate into the tunic, while others remain in the subepidermal region to perform other functions.

By 36 hr. (Fig. 11) the epidermal outpushing has increased in length and, in the subepidermal space, all stages in the differentiation of the haemoblasts into connective tissue cells can be distinguished (Fig. 11). The many cells which have fully differentiated have begun to secrete the connective tissue matrix (Fig. 11) characteristic of the subepidermal regions of the normal mantle.

It is to be remarked that very rarely in the regenerating regions do the different types of blood cell differentiate from lymphocytes. Differentiation almost invariably appears to involve haemoblasts alone. Pérès has stated (1943) that the various blood elements, in young animals differentiate from haemoblasts, in older animals from lymphocytes. It would therefore appear that in old specimens of *Ciona* regeneration is associated with the type of cell differentiation characteristic of young animals.

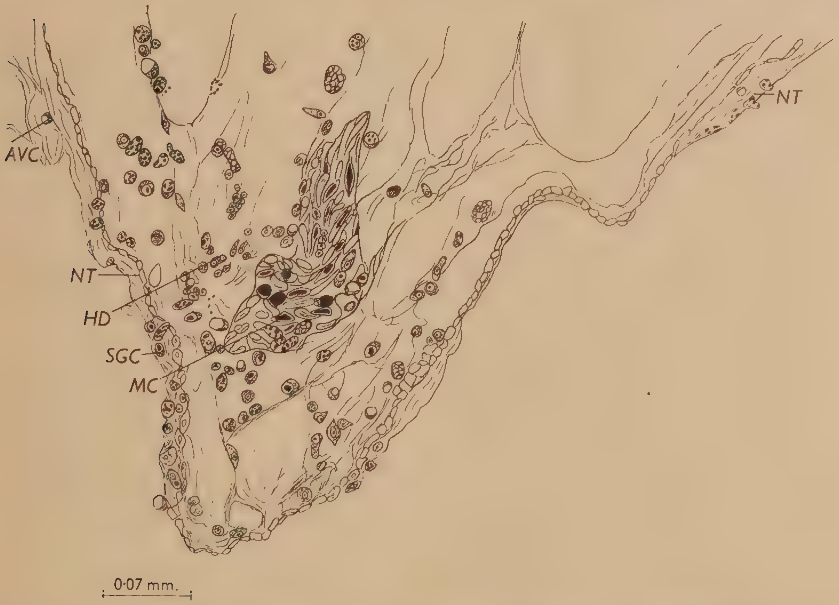

Fig. 12. Fifty-six hours—mouth. *AVC*, acidophil vacuole cell; *HD*, dividing haemoblast; *MC*, muscle cell; *NT*, new tunic; *SGC*, cell with single granule.

In one specimen killed 36 hr. after operation, the inhalant siphon had been cut sufficiently far posteriorly to involve the anterior pharynx. Here regeneration of new gill bars was in process. It was of interest to note that the endoderm and ectoderm, separated by connective tissue, which lined the anterior end of the pharynx, were identical in appearance. The ectoderm and endoderm had joined round the cut edge, and could not be distinguished from each other. Laterally and internally, slight bulges were visible at intervals, the forerunner of new gill bars and papillae.

By 48 hr. the ectodermal outpushings at the apices of the regenerating siphons have become even longer. In the posterior region of the regenerated zone a clumping of haemoblasts in the recently secreted connective tissue matrix has occurred. Differentiation of these cells along three lines has already been initiated. One group has begun slightly to elongate to form new muscle cells, another to differentiate into cells with refringent granules, and a third, much smaller than the others, consists of haemoblasts in the process of differentiation into cells with a single refringent granule. Cells with several refringent granules appear frequently to be associated with muscles. The reason for the association is not known. Further changes in muscles damaged as a result of the operation, chiefly the longitudinal muscles of the mantle, can now be distinguished. Not merely are the slightly damaged cells now intact and growing in length, but in the spaces left after the removal of badly damaged cells, haemoblasts and cells with refringent granules are found.

In the test where new growth is occurring the cells present are mostly those with refringent granules; otherwise the situation is little different from that at 24 hr., save that by now the cut edge is regular in outline.

Up to this time no new secretion of the tunic by the newly differentiating mantle has occurred, but by 56 hr. the first appearance of a very delicate tunic round the basal regions of the new growths can be distinguished (Fig. 12). Embedded in this test are cells of all three types described by Pérès, as participating in the regeneration of a new tunic. In general, the majority are those with many refringent granules; those with a small acidophil vacuole are in the minority.

In the subepidermal areas muscle differentiation continues and additional groups of haemoblasts which later give rise to muscle cells can be observed (Figs. 12–13). Also associated with the newly differentiated muscles are cells in which many small vacuoles are present. In some only one or two granules are present, in others they are entirely lacking. These are probably stages in the degeneration of the refringent granule cells.

In certain regions of the newly regenerating siphons there appears to have been no appreciable growth in length between 48 and 56 hr.; in others growth has continued. The third day sees the appearance of lobes characteristic of the siphons; and the differential growth just mentioned is undoubtedly associated with the development of these lobes. This type of growth continues through the third day.

By 60 hr. the picture of the regenerative processes is very little different from that observed at 56 hr. In the new tunic, which by now has here and there fused with the old, the number of cells with single acidophil vacuoles has increased, and some of the cells with many refringent granules have entered into the second phase of activity (Pérès, 1948*a*). The original granules are much paler and, in some cells, difficult to distinguish, while densely staining spheroidal masses, probably proteins, are appearing.

From 76 hr. until the end of the fourth day (Figs. 14–16) the regeneration of the siphons continues by repetition of the processes already described. For the first time near the outer edge of the tunic secreted by the newly regenerated mantle, a few cells with large vacuoles whose contents stain with toluidine blue, methyl green, and are positive to Feulgen, are seen.

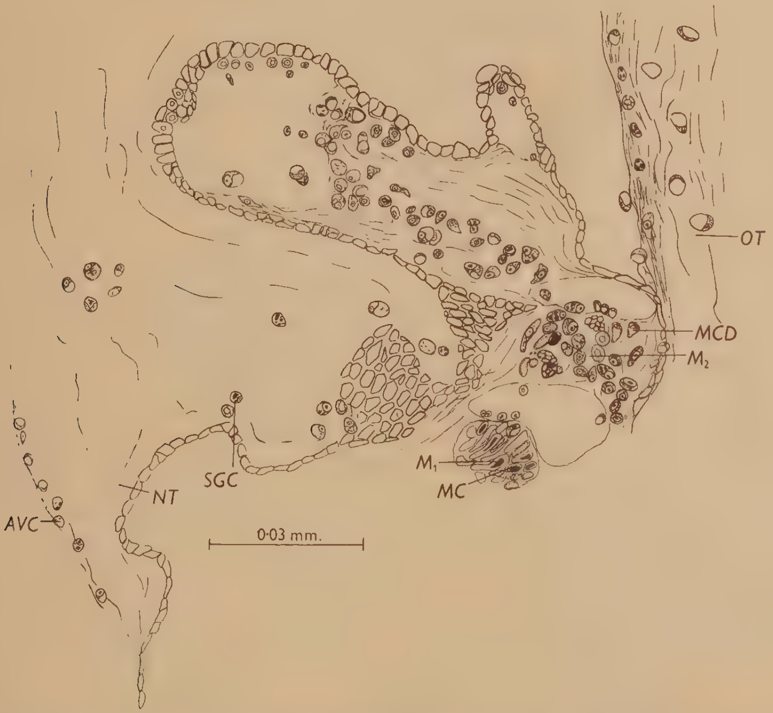

Fig. 13. Sixty hours—atriopore. *AVC*, acidophil vacuole cell; M_1, almost completely differentiated muscle cells; M_2, younger cells differentiating into muscle cells; *MC*, muscle cell; *MCD*, differentiating muscle cell; *NT*, new tunic; *OT*, original tunic; *SGC*, single granule cell.

The peculiar nature of the muscle cells should now be mentioned. Sections through individual fibres of fully developed muscles show that thymonucleic acid is evenly distributed throughout the cell, positive results having been given by both the Feulgen test and Unna's pappenheim. During the development of a fibre from a haemoblast the thymonucleic acid in the cytoplasm steadily increases in amount. With its appearance the small nucleus present during the earlier stages of growth ceases to be microscopically recognizable. The fully differentiated muscle cell is therefore apparently non-nucleated.

The description of the regeneration processes just given is characteristic of the majority of animals examined. There are minor variations in the time of tunic secretion by the newly formed mantle. It may be slightly earlier than has been stated.

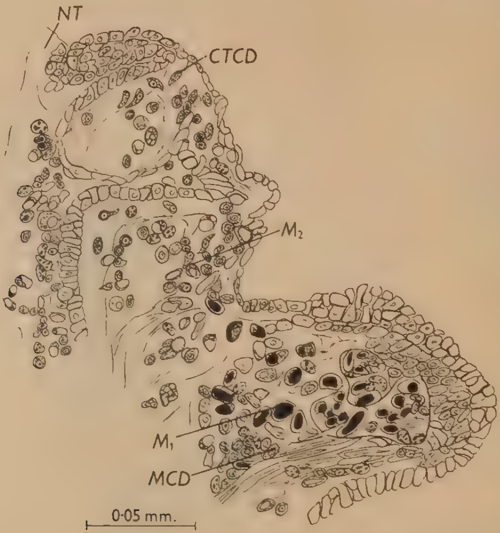

Fig. 14. Seventy-six hours—mouth. *CTCD*, differentiating connective tissue cell; M_1, almost fully differentiated muscle cells; M_2, younger cells differentiating into muscle cells; *MCD*, differentiating muscle cell; *NT*, new tunic.

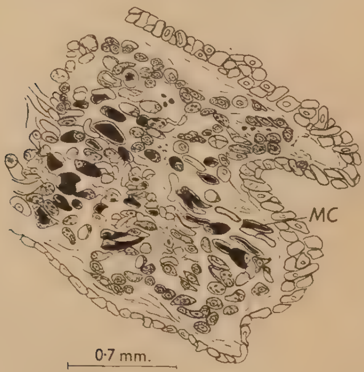

Fig. 15.

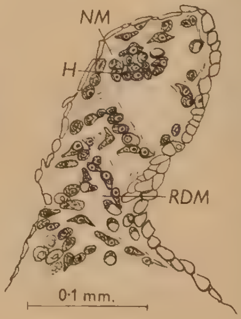

Fig. 16.

Fig. 15. Eighty hours—mouth—further growth. *MC*, muscle cell.

Fig. 16. Eighty hours—atriopore—continued growth. *H*, haemoblast; *NM*, new connective tissue matrix; *RDM*, recently differentiated matrix.

Discussion

The regenerative processes of specimens of *Ciona intestinalis* from which the siphons have been removed involve the haemoblasts, ectoderm, and a small region of the pharyngeal endoderm. The operation stimulates division and migration of the haemoblasts to such an extent that in a few hours a large number will have appeared at the site of the injury. Once there, they may differentiate directly into connective tissue cells, muscle cells and into various types of blood cell, or, much less frequently, differentiation of the blood cells may be preceded by a division of the haemoblast into two lymphocytes. The ectoderm and endoderm of certain regions of the pharynx are microscopically indistinguishable, and in some animals from which the most anterior region of the pharynx has been removed it is impossible to tell whether ectoderm or endoderm is proliferating to give rise to the new endoderm. The participation of haemoblasts in the formation of the new epithelia is in doubt. Certainly in some sections haemoblasts are seen wedged among the new ectoderm and endoderm cells. They may, however, be merely in transit through the epithelium.

There have been many accounts of asexual reproduction and regeneration among ascidians, but almost all of them suffer from inadequate accounts of the histological processes involved. Nevertheless, an attempt will be made to review the processes, to relate the phenomena of siphon regeneration in *Ciona* to those of ascidian regeneration in general, and to formulate certain principles which may go some way towards explaining the outstanding powers of asexual reproduction and regeneration in ascidians.

Asexual reproduction and regeneration in the phlebobranchs and aplousobranchs (in Harant's classification, 1933) either involve internal organogenesis from the epicardium, a pharyngeal outgrowth, or from a mesenchymatous septum, or from the blood cells, as in *Ciona*. As will be seen later both organogenesis from the epicardium and from the septum can occur in one and the same animal.

Before these methods can be discussed, more detailed descriptions of the processes involved must be given.

Asexual reproduction in *Diazona violacea* involves the formation of buds by transverse constrictions of the post-abdomen (Berrill, 1948 *b*). Each bud contains ectoderm, muscles, two sections of the alimentary canal and part of the epicardium. From the epicardium are proliferated cells from which the pharynx, peribranchial sacs, cerebral ganglion and the anterior part of the post-pharyngeal alimentary canal of the new individual develop. The pericardium also has its origin in this structure. The two sections of the parent alimentary canal unite posteriorly and the loop so formed increases in length. This account lacks any detailed histology.

However, Brien's (1933) work on *Archiascidia neapolitana* (Julin) may clarify the picture. When the thorax has been naturally or artificially lost, the ventral wall of the epicardium thickens and proliferates cells, described by Brien as embryonic in appearance, and it is from these cells that the two pharyngeal lobules develop that later coalesce to give the definitive pharynx. Significantly Brien describes the cells proliferated by the epicardium as 'embryonic', and very probably these are haemoblasts. In 1933 Pérès had not described the various types of blood cell to be found in ascidians and this term was therefore unknown to Brien. As noted above, Berrill (1948 *b*) describes the epicardium in *Diazona* as dividing to give rise to cells from which the branchial sac, atrium, etc., develop. These cells may well be similar to those described as embryonic in *Archiascidia* and thus also be haemoblasts.

Asexual reproduction in *Clavelina phlegrea* (Salfi) is in its major aspects (Brien, 1930) an almost exact replica of that of *Archiascidia neapolitana*, but in another species, *Clavelina lepadiformis*, and in the Perophoridae, an apparently different process is involved. Here each of the bud chambers that develop upon a stolon contains a 'blastogenic mass', consisting in *C. lepadiformis* of a mass of blood cells, which Brien (1930) calls lymphoblasts, and of mesenchyme cells. These cells are the products of the disintegration of a mesenchymatous septum to which the lymphoblasts had become attached. In this mass an 'endoblastic vesicle' appears, on the future dorsal side of which there develops a neurogenital 'massif'. This vesicle is the forerunner of the pharynx peribranchial cavities, epicardium and the remainder of the alimentary canal. The neural and genital cells dissociate from each other. Further development consists of the differentiation of organs whose 'anlagen' have now appeared.

It therefore seems that, according to the current view, two methods of asexual reproduction and regeneration can occur in the one genus *Clavelina*. Further, it has been found experimentally that the two processes can occur in the same species *C. lepadiformis*. Asexual reproduction is normally at the expense of a mesenchymatous septum. If, however, as a result of transverse cuts, a bud chamber is formed in the oesophageal region, the new organs develop from the epicardium (Brien, 1933; Berrill & Cohen, 1936). The epicardium, a pharyngeal outgrowth, is generally said to be an endodermal organ. Thus, clearly, according to current ideas, two very different methods of asexual reproduction can occur, not merely in the same genus, but even in the same species. This conclusion seems to be very much open to doubt.

As already stated, the epicardium is generally regarded as an endodermal structure. Berrill (1951) has, however, suggested that it may possibly be of mesodermal origin. There are now good reasons for the acceptance of this idea. Enterocoely is common among protochordates and echinoderms, possible ancestors or close relations to the ancestors of the urochordates. To suggest, therefore, an enterocoelic origin for the epicardial tubes seems not unreasonable. This would make the cavity of an epicardial tube a portion of the coelom. In its continued growth, in *Ciona* the epicardium comes to invest the abdominal organs; the potential cavity in this double-layered structure would thus form a perivisceral coelom, whose absence in ascidians has seemed anomalous. Further, the epicardium has been stated by Pérès (1947*c*) to be the centre of haemoblast proliferation, particularly in larval life. He also notes that in the Polyclinidae it undergoes haemoblastogenesis in the adult condition. In cephalochordates and in most vertebrates, according to Brachet (1944), blood cells are of mesodermal origin.

If the mesodermal nature of the epicardium be conceded, then epicardial and septal budding are but different manifestations of a single process. This hypothesis, however, does not account for the phenomena of siphon regeneration in *Ciona* and an attempt will now be made to show that this apparent defect can be overcome.

Pérès (1947*c*) pointed out the epicardial origin of haemoblasts, and Brien (1933) noted the proliferation of embryonic cells from the epicardium during asexual reproduction in *Archiascidia neapolitana*. He also described the mesenchymatous septum in *Clavelina lepadiformis* as consisting, very largely, of lymphoblasts (haemoblasts). It might also be suggested that the already differentiated mesenchymatous cells would be unlikely to play an important role in organogenesis. Thus, in both species the same cells, the haemoblasts, are almost certainly extensively involved in asexual reproduction, and in both they have the same origin, the epicardium. Berrill's (1948*b*) account of asexual reproduction in *Diazona violacea* offers no serious objection to this view. The epicardial origin of cells involved in organogenesis is noted. It is true that he states that blood cells play no part in the process, but this statement refers to the nutritive trophocytes.

It therefore seems reasonable to suggest that, in the phlebobranchs and aplousobranchs, asexual reproduction occurs at the expense of haemoblasts, cells proliferated in an undifferentiated condition, from the epicardium. The position of *Ciona*, a phlebobranch, in so far as regenerative processes are concerned, is now clear. In this animal, the cells responsible for the regeneration of all tissues other than the epithelia, of the siphons, are haemoblasts originating in the epicardium.

As a point of interest, according to Pérès (1947*b*), the cerebral ganglion of *Ciona* differentiates from neoblasts. These cells appear to be very like haemoblasts. The cerebral ganglia of *Diazona* and *Clavelina*, if the views outlined above be accepted, also differentiate at the expense of haemoblasts.

Finally, a third method of asexual reproduction in ascidians is seen in the pallial budding of the stolidobranchs. Here mesenchyme and ectoderm alone are involved in organogenesis; the mesenchyme is that tissue lying between the two layers of ectoderm of the outer atrial wall. The vesicle that gives rise to the future pharynx, nerve complex and atrial cavity is lined, at its first appearance, by ectoderm. A neural tube and two peribranchial cavities evaginate and become isolated from the parent vesicle, after which process differentiation to form the definitive organs takes place. The future genital cells are carried from the parent gonads to the bud by the blood stream (*Botryllus*), or differentiate from mesenchyme cells, *in situ* (*Heterocarpa glomerata*). It must be emphasized that detailed histological investigations of the processes involved have never been attempted, and further work along these lines may considerably modify our present information.

I would like to express my most grateful thanks to the Director of the Marine Biological Laboratory at Plymouth, Mr F. S. Russell, F.R.S., for having afforded me the facilities of the Laboratory where all the observations upon the living material were made and much of the work upon the preserved material was carried out. I would also like to express my gratitude to Mr C. C. Hentschel of Chelsea Polytechnic and to Prof. P. B. Medawar, F.R.S., of University College, London, for their most helpful suggestions and criticisms of the manuscript. Finally I wish to acknowledge the most valuable assistance of the University of London Grants Committee, from whom I have on loan a Dixon Fund Microscope without which the work could not have been completed.

SUMMARY

Regeneration of the siphons of normal specimens of *Ciona intestinalis* has been found to be completed, in every particular other than that of length, in 4 days.

Removal of the neuroglandular complex retards the regeneration rate.

With the exception of the epidermis and of the basic tunic, the new structures differentiate from haemoblasts.

A tentative synthesis has been made of the information available upon asexual reproduction and regeneration in the Ascidiaceae.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

An Experimental Study of the Scattering of Light by Natural Waters

By **W. R. G. Atkins** and **H. H. Poole**

Proc. Roy. Soc. Lond., B, Vol. 140, 1952, pp. 321–38

A blue-sensitive multiplier phototube was used to measure light scattered from a parallel beam in distilled, tap and sea water, the first-named serving as a check upon errors from extraneous sources of light. Forward and back scatter are closely the same for distilled water, but with natural waters by far the greater part of the effect occurs through angles less than 25°. A minimum is found for a deviation of about 110°, back scattering increasing somewhat for greater angles. The relative importance of forward scatter increases with turbidity, and in sea water about three-quarters of the effect is due to matter removable by filtration through a collodion filter of average pore diameter 1 μ or by sedimentation; further passage through 0·6 and 0·2 μ filters produces little change. Scattering is greater in blue light. Plymouth tap water scatters more than surface coastal water and the latter more than surface water 20 miles out, station E 1. Surface water scatters more than deeper—the water column being remarkably homogeneous even when a well-marked thermocline had existed for weeks, but a small increase was detectable at the top of the cold water. E 1 surface water increased in scattering between August and January, and decreased till May. Deep water showed little change. Extinction due to scattering between 20 and 155° amounted to less than one-sixth of that found for a similar sample with a Pulfrich photometer, so probably much scattering occurs below 20°. This explains why Pulfrich extinctions are so much greater than vertical extinction coefficients found in the sea.

The preponderance of forward scattering within the range 20–155° and the effects of filtration suggest that such scattering is due chiefly to refraction through transparent mineral particles, large compared with the wave-length of light. The refractive index of organic matter is too near that of water to produce refraction through angles as large as 20°. Such matter may, however, be responsible for some of the scattering through smaller angles, which apparently accounts for most of the turbidity found with the Pulfrich photometer. W.R.G.A.

D-AMINO-ACID OXIDASE IN THE MOLLUSCAN LIVER

By H. Blaschko and Joyce Hawkins

Biochem. Journ., Vol. 52, 1952, pp. 306–12

The livers of two cephalopods, *Octopus vulgaris* and *Eusepia officinalis*, contain the enzyme D-amino-acid oxidase. Since this is the first time that the enzyme has been described in invertebrates, some of its properties have been studied and compared with those of the corresponding mammalian enzyme. The pattern of substrate specificity of the cephalopod enzyme is similar to the mammalian enzyme, but there are a few differences; for instance, the octopus enzyme oxidized D-glutamic acid more rapidly. Both homogenates and acetone-dried powders were active.

The enzyme was also found in *Mytilus edulis* and in *Helix*, but not in *Anodonta*. H.B.

OBSERVATIONS ON AMINE OXIDASE IN CEPHALOPODS

By H. Blaschko and Joyce Hawkins

Journ. Physiol., Vol. 118, 1952, pp. 88–93

This paper contains a report on the occurrence of the enzyme amine oxidase in the tissues of *Octopus vulgaris* and *Eusepia officinalis*.

In *Octopus*, very high amine oxidase activity was found in liver extracts; in order of decreasing activity, the enzyme was present in the posterior salivary glands, the anterior salivary glands and in brain tissue. The high amine oxidase activity of the tissues of *Eusepia* already described in earlier papers has been confirmed. Differences in the pattern of substrate specificity between the enzymes from the two species are also reported.

The significance of these findings in relation to the presence of amines in the tissues of cephalopods is discussed.

Amine oxidase activity was not detected in *Mytilus edulis* and *Helix*.

H.B.

THE MECHANICAL ANALYSIS OF THE RESPONSES FROM THE END-ORGANS OF THE HORIZONTAL SEMICIRCULAR CANAL IN THE ISOLATED ELASMOBRANCH LABYRINTH

By J. J. Groen, O. Lowenstein and A. J. H. Vendrick

Journ. Physiol., Vol. 117, 1952, 329–46

The mechanical properties of the cupula-endolymph system were subjected to experimental tests by means of the oscillographic method of recording from the nerve supplying the horizontal semicircular canal of the isolated labyrinth of the ray (*Raja clavata*).

Tests on torsion-swing and turn-table furnished conclusive evidence that the cupula-endolymph system behaves like a true pendulum, and a differential equation describing the behaviour of this system is suggested.

During the tests, sense organs deviating from the expected mode of behaviour were encountered, but a simple physical model (the characteristic curve of an electronic valve) renders possible a unified description of all behaviour types of semicircular canal organs. O.L.

MUSCLE ACTIVITY AND DRUG ACTION IN THE BODY-WALL OF THE SABELLID WORM *BRANCHIOMMA VESICULOSUM* (MONTAGU)

By J. A. Colin Nicol

Physiol. Comp. et Oecol., Vol. 2, 1952, pp. 339–45

The effects of certain autonomic drugs on strips of body-wall of *Branchiomma vesiculosum* (Sabellidae) are described. In preparations lacking central nervous system, acetylcholine and nicotine cause contractions of the longitudinal musculature. The threshold concentration lies about 1/100,000, and nicotine has the stronger effect. Eserine and DFP potentiate the contraction due to acetylcholine. Atropine, pilocarpine, and adrenaline are without effect. Repetitive electrical stimulation of body-wall (with and without nerve cord) shows no augmentation of response after eserinization. D-tubocurarine does not black neuromuscular transmission in *Branchiomma*. The contrast with the earthworm preparation in which augmentation or facilitation occurs is brought out. Evidence for antagonistic systems, adrenergic and cholinergic, in annelids is reviewed, and the differences in the blocking action of curare within this group are taken to indicate considerable diversity in receptor mechanisms concerned with neuromuscular transmission.

J.A.C.N.

J. Mar. biol. Ass. U.K. (1953) 32, 273–288.
Printed in Great Britain

STUDIES IN THE PHYSIOLOGY OF COMMENSALISM. IV. THE POLYNOID GENERA *POLYNOË*, *LEPIDASTHENIA* AND *HARMOTHOË*

By Demorest Davenport

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The role of chemical substances released by hosts in governing the behaviour and determining the specificity of commensal polynoids has been demonstrated in previous investigations (Davenport, 1950, 1953; Davenport & Hickok, 1951). In all associations so far investigated, in which the host is an echinoderm, the commensals readily give a powerful positive response to their hosts either remotely or on contact, even after the partners have been kept under laboratory conditions for some while.

More difficulty has been experienced in demonstrating such a response in polynoids commensal with other annelids. *Halosydna brevisetosa*, commensal with *Amphitrite robusta* in Puget Sound, appears to be unable to discern the presence of its host at any distance but responds when it comes in contact with the host. Using *Gattyana cirrosa* and *Lepidasthenia argus*, commensal respectively with *Amphitrite johnstoni* and *A. edwardsi*, the author (1953) was unable with the Y-tube choice-apparatus of the 1950 and 1951 experiments to observe any indication of a sensitivity to water coming from aquaria containing hosts, even when a large number of fresh hosts were placed in the test aquarium. Using *Gattyana* which had been housed for some time with their hosts in glass U-tubes the author was unable to repeat previous observations made and reported to him by R. Phillips Dales, who, using freshly collected material, observed responses by these commensals to tentacle and body of their host, as well as to water drawn from the vicinity of the host's body by a pipette.

It became apparent that a thorough investigation of a terebellid-polynoid partnership required a continuous supply of fresh material, available regularly and not merely at the infrequent periods of low spring tides. The discovery of such a supply at Plymouth made the following experiments possible.

These investigations are a continuation of studies made during the winter of 1953 at the Plymouth Laboratory under a grant from the John Simon Guggenheim Memorial Foundation. The author is indebted to the Foundation and the Director and Staff of the Plymouth Laboratory for their support and co-operation.

POLYNOË SCOLOPENDRINA SAVIGNY

Materials and Methods

On the Eddystone grounds great masses of intertwined *Chaetopterus* tubes may readily be dredged or trawled. The fauna occurring in and among these is rather diverse and worthy of detailed study and description. Here, making its rather characteristic tubes of sandy gravel mixed with bits of shell, between inhabited and in abandoned *Chaetopterus* tubes, occurs the handsome strawberry-coloured terebellid *Polymnia nebulosa* (Montagu). About 30% of these are accompanied by the commensal *Polynoë scolopendrina* Savigny, which, if large, often lies head-to-head along the body of the host or, if small, may be found wrapped about the extreme anterior region of the terebellid's body.

Although the *Plymouth Marine Fauna* (Marine Biological Association, 1931) gives no other host for this polynoid, Darboux (1899) states that at Wimereux the commensal is taken not uncommonly in the tubes of *Lanice conchilega* from deep water. McIntosh (1900) states that the polynoid occurs in the Channel Islands not only with *Polymnia* but also 'between chinks of rock (gneiss) in muddy sand in the tubes of' the eunicid *Lysidice ninetta.* It is of interest that in one locality, at least, the commensal occupies the tubes of two relatively distantly related annelids of different families, when it has not been recorded, so far, from more than two of the numerous terebellid species of Channel waters.

The technique of determining responses of the polynoids was very similar to that used in the analysis of the responses of *Acholoë astericola.* A number of commensals were placed in individual dishes in filtered 'outside' sea water. Materials to be tested were presented to the head of each commensal in rapid order and the response recorded. Between each series of tests the commensals were washed in clean sea water and the instruments in alcohol acetone.

At the very outset, brief experiments indicated that a commensal responded in a typical way if the host was brought in close proximity to, and in contact with, the commensal's head. The polynoid responded by orienting its head squarely in the direction of the host and by actively moving closer to it. If the body of the host was kept stationary and the commensal came in contact with an extended tentacle, it very quickly followed up the tentacle and brought itself alongside or underneath the host's body. If the body of the host were moved in any direction, the commensal would usually attempt to maintain contact no matter in what direction movement occurred.

Responses were recorded as follows. If the presentation of material at the head of the polynoid and subsequent movement of the material resulted in an immediate and active effort to follow it, the response was recorded as +. If the response was delayed or the activity produced was sluggish, this was recorded as (+). No response whatever after repeated presentation was recorded as 0.

Observations

Experiment No. 1. How specific is the response? Will the commensals respond to the presence of any polychaete if it is presented at their head? Members of four genera of polychaetes immediately available at the Laboratory were tested, with these results:

Worm	*Chaetopterus variopedatus*	*Arenicola marina*	*Myxicola infundibulum*	*Sabella pavonina*	Host control
1	o	o	o	o	+
2	o	o	o	o	(+)
3	o	o	o	o	+
4	o	o	o	o	o
5	o	o	o	o	+
6	o	o	o	o	+
7	o	o	o	o	+
8	o	o	o	o	+

It is clear that the commensals do not respond indiscriminately to polychaetes and that the host is 'recognized'. It is of interest that the commensals show no response whatever to *Chaetopterus*, with which in the particular environment from which the test sample came they are rather intimately associated.

Experiment No. 2. How specific is the response within the family Terebellidae, to which the host *Polymnia* belongs? A number of terebellids which were readily available at the time were tested:

Worm	*Amphitrite edwardsi*	*Terebella lapidaria*	*Polycirrus caliendrum*	Host control
1	o	o	o	+
2	o	o	o	o
3	o	o	o	+
4	o	o	o	+
5	o	o	o	+
6	o	o	o	(+)
7	o	o	o	+
8	o	o	o	+

Worm	*Amphitrite johnstoni*	*Amphitrite gracilis*	Host control
9	o	o	+
10	o	(+)	+
11	o	+	+
12	+	+	+
13	(+)	o	+
14	o	o	+
15	o	(+)	+
16	(+)	(+)	+

It would appear from these data that the response is not absolutely specific, since the *Polynoë* will occasionally respond to *Amphitrite johnstoni* and *gracilis*. Neither of these terebellids occur commonly in the same environment as the normal host.

Experiment No. 3. How do the commensals of *Polymnia* behave towards the two other polychaetes that have been recorded as their host, the terebellid *Lanice conchilega* and the eunicid *Lysidice ninetta*?. These were tested:

Worm	*Lanice conchilega* (Terebellidae)	*Lysidice ninetta* (Eunicidae)	Host control
1	0	(+)	+
2	0	(+)	+
3	0	+	+
4	0	+	+
5	0	+	+
6	0	0	+
7	0	(+)	+
8	0	+	+

The tests with *Lanice* were repeated with fresh material

Worm	*L. conchilega*	Host control
9	0	+
10	0	+
11	0	+
12	0	0
13	0	+
14	0	+
15	0	+

That the population from *Polymnia* does not respond to *Lanice* (recorded as an alternate host in France) seems to indicate that the attractants to which the *Polymnia* and *Lanice* populations are adapted to respond are not identical. It is possible, however, that the host-record for *Lanice* may be the result of a mistaken identification; *Lanice* is common enough in Plymouth waters but the association has never been observed there.

The significance of the strong response to the eunicid *Lysidice* is discussed in a further paragraph.

What can be determined concerning the manner in which the response is elicited and the nature and source of the attractant?

Experiment No. 4. *Polynoë* gives a marked response to host tentacle. Does simple physical contact with any terebellid tentacle initiate a response? Isolated tentacle from a form to which, when whole, *Polynoë* does not respond was tested:

Worm	*Amphitrite edwardsi* tentacle	Host tentacle
1	0	+
2	0	+
3	0	+
4	0	+
5	0	+
6	0	+
7	0	+
8	0	+

Contact with any tentacle does not initiate responses. Host tentacle is 'recognized'.

Experiment No. 5. In the above experiments it has been observed that the commensals are attracted by both the body and tentacle of their host. However, one may often find the *Polynoë* at some distance from the host within the host's tube. The tube of *Polymnia* is manufactured much as that of other terebellids by a secreted cement which knits together sandy gravel and bits of shell. Is the commensal 'bound' by the presence of attractant to the tube itself?

Bits of tube were presented at the heads of commensals:

Worm	Tube	Host control	Worm	Tube	Host control
1	(+)	+	9	0	+
2	(+)	+	10	0	0
3	+	+	11	(+)	(+)
4	(+)	+	12	0	+
5	+	+	13	+	+
6	(+)	+	14	(+)	+
7	+	+	15	(+)	+
8	(+)	+	16	(+)	+

These experiments indicate that enough attractant may be present in the host's tube to elicit sluggish or delayed and occasional full responses. Although care was taken in these experiments to remove bits of living tentacle from the pieces of tube, it is possible that bits were present and that responses were elicited by them, for occasionally pieces of tube which had unquestionably not long before been occupied by the host failed to elicit responses from any of eight commensals. Though these results are not entirely uniform, it appears that attractant may be present in fresh tube material, perhaps in reduced quantity or in an altered state.

Experiment No. 6. The body of the host is coated with transparent mucus. Is attractant present in it? When freed from its tube in a dish of clean sea water *Polymnia* produces mucus that may be richly taken up in cotton-wool. Such mucus-soaked cotton-wool was presented at the heads of commensals:

Worm	Cotton-wool plus mucus	Cotton-wool control	Host control
1	0	0	0
2	0	0	+
3	0	0	+
4	0	0	+
5	0	0	+
6	0	0	+
7	0	0	+
8	0	0	+

The data indicate that attractant is either not present in the mucus or that not enough is present in it to elicit even delayed responses.

Experiment No. 7. What can be determined concerning the source of attractant by dissection techniques? A large *Polymnia* was dissected and a number of tissues isolated and washed in clean 'outside' sea water. These were presented:

Worm	Host control	Gonad	Gut and blood	Gill	Ventral shield	Tip of abdomen
1	+	o	o	+	o	o
2	+	+	(+)	+	(+)	(+)
3	+	o	o	+	(+)	o
4	+	o	o	+	+	+
5	+	o	o	o	+	+
6	+	o	o	+	o	o
7	+	o	(+)	+	+	+
8	+	o	o	+	+	+

Exp. no. 4 has already shown that host tentacle elicits a powerful response. The above experiment indicates that gill is also very effective. The method of assay by means of responses is obviously not precise enough for comparisons between different tissues. However, it is clear that the internal organs gave little evidence of the presence of attractant (positive responses may have been the result of contamination in removal), while all the external tissues indicated the presence of enough attractant to elicit a number of full responses.

Experiment No. 8. In preliminary experiments to establish the regularity of response to host the commensals were occasionally observed to orientate their head toward the host when the host was not more than a mm. or so distant from it. In addition, Dales had reported (personal communication) that he had elicited responses from *Gattyana cirrosa* to water drawn with a pipette from a point close to the host's body. Can the presence of attractant in the immediate vicinity of the host be demonstrated consistently when contact with the host's body is effectively prevented?

An entire host was placed in a small sac of 200-mesh bolting silk and tied off. Great care was taken to prevent contamination of the outside of the sac by material from the host. The sac was presented:

Worm	Sac	Host control	Sac control
1	(+)	+	o
2	+	+	o
3	(+)	+	o
4	+	+	o
5	+	+	o
6	o	+	o
7	o	(+)	o
8	(+)	o	o

Evidence is presented by these data that a sufficient quantity of attractant diffuses a short distance from the host's body to be perceived by the commensal, since the small size of the mesh makes it unlikely that any direct contact with host tissue occurred.

Experiment No. 9. Can attractant be demonstrated in ground-up whole host? An entire fresh host was ground up in 3 ml. of clean 'outside' sea water and sand. Bits of cotton-wool soaked in this preparation were presented:

Worm	Preparation	Cotton-wool control	Host control
1	0	0	+
2	0	0	+
3	0	0	+
4	0	0	+
5	0	0	+
6	0	0	+
7	0	0	+
8	0	0	+

It thus appears that in the course of this preparation attractant is either destroyed or masked by the presence of injury substances.

The above experiments on the nature and source of the attractant indicate that the substance to which the polynoid responds is released and is present on the external surface of the host's body and may perhaps be involved in tube manufacture. That it is either unstable or very closely bound to other materials and hence released in such small quantity as to be rapidly diluted below the threshold of sensitivity of the polynoid is indicated by the fact that near contact is necessary for it to take effect. Destruction of the effect by grinding may be an indication of instability.

Work at present in progress indicates that it may be possible to learn more about the nature of some terebellid attractants by using tube material. *Polymnia* tube material, for some undetermined reason, does not always give constant results; attractant may be present in some parts of the tube and not in others. However, it has been possible to demonstrate that effective pieces of tube may retain their attraction for as long as 15 hr. after removal from the host if kept in cool circulation. Brief experiments have also indicated that temperatures as high as 60° C. destroy the attractiveness of tube material. One may be permitted to suspect that the attractant is a specific mucoprotein, but further investigations must obviously be carried out before this can be demonstrated.

LEPIDASTHENIA ARGUS HODGSON

From previous tests (Davenport, 1953) it was concluded that this handsome polynoid, commensal with the terebellid *Amphitrite edwardsi* which is its only known host, did not appear to be able to discern the presence of its host when tested in the Y-tube choice-apparatus of the 1950 experiments. However, experiments conducted on material freshly obtained with the aid of the Plymouth Easter Classes from the flats of Salcombe Estuary showed that these polynoids exhibit the same response when in close proximity to or touching their host as does *Polynoë scolopendrina* to *Polymnia nebulosa*. How specific is this response?

Experiment No. 1. Two polychaetes were tested which may be taken in the same immediate environment as the partnership:

Worm	*Arenicola marina*	*Myxicola infundibulum*	Host control
1	o	o	+
2	o	o	+
3	o	o	+
4	o	o	+
5	o	o	+
6	o	o	+
7	o	o	+
8	o	o	+

Experiment No. 2. Tests were made with different members of the host's family, Terebellidae:

Worm	*Polymnia nebulosa*	*Terebella lapidaria*	*Amphitrite gracilis*	*Amphitrite johnstoni*	Host control
1	o	o	o	o	+
2	o	o	o	+	+
3	o	o	o	o	+
4	o	o	o	+	+
5	o	o	o	(+)	+
6	o	o	o	o	+
7	o	o	o	o	+
8	o	o	o	o	+

The response appears to be relatively specific within the family of the host. As might be expected, an occasional response was shown to *Amphitrite johnstoni*, which is closely related to the host. Such responses were exhibited by the large and active commensals as a movement directly to the body of *A. johnstoni*, followed by restlessness and a tendency to move away.

Both *A. edwardsi* and *A. johnstoni* occur at Salcombe. *Lepidasthenia* has never been taken with *Amphitrite johnstoni*, which does not appear to attract the polynoid strongly enough for an association to be established.

HARMOTHOË SPINIFERA (EHLERS)

An investigation of the notes of the late Prof. J. H. Orton, F.R.S., now in the possession of Dr D. P. Wilson, disclosed that he had found a polynoid, which for want of accurate identification he labelled 'Polynoid B', in association with *Amphitrite gracilis* and *Polycirrus* (*caliendrum*?) at Rum Bay. The former association was re-discovered by the author during the 1953 Easter Class trips to Wembury and later at Mount Edgecumbe in Plymouth Sound. The animals can be obtained at medium low water by cracking apart the exposed soft layers of red shale; they appear to have somewhat the same ecological requirements as the eunicids *Marphysa* and *Lysidice*, often found near them. The polynoids were identified by Mr G. M. Spooner and the author as *Harmothoë spinifera* (Ehlers) in Fauvel (1923). With their hosts they have been preserved and placed in the Laboratory Museum.

Since the *Amphitrite gracilis–Harmothoë spinifera* partnership may be the most readily available intertidal terebellid-polynoid association in Plymouth waters, it seemed advisable to seek information concerning this polynoid's specificity of response.

Experiment No. 1. Do the commensals respond to the two polychaetes other than their host that occur commonly in the immediate red-shale environment? Two species were tested:

Worm	*Lysidice ninetta*	*Marphysa sanguinea*	Host control
1	0	0	(+)
2	0	0	+
3	0	0	+
4	0	0	+
5	0	0	+
6	0	0	(+)

Experiment No. 2. How specific is the response within the host family, Terebellidae? Four species were tested:

Worm	*Polymnia nebulosa*	*Terebella lapidaria*	*Amphitrite edwardsi*	*Amphitrite johnstoni*	Host control
1	0	0	(+)	(+)	+
2	0	0	0	0	+
3	0	0	0	0	+
4	0	0	(+)	(+)	+
5	0	0	0	0	+
6	0	0	(+)	0	+
7	(+)	(+)	0	0	+
8	0	0	(+)	(+)	+
9	0	0	(+)	0	+
10	0	(+)	(+)	(+)	+

Experiment No. 3. How does the commensal population from *Amphitrite gracilis* behave toward the alternate host *Polycirrus caliendrum*?

Worm	*Polycirrus caliendrum*	Host control
1	+	+
2	0	(+)
3	(+)	+
4	+	+
5	(+)	+

Repeated with a second test sample and control negative:

Worm	*Polycirrus caliendrum*	*Polymnia nebulosa* (control negative)	Host control
1	0	(+)	+
2	+	0	+
3	+	0	+
4	+	0	+
5	+	0	+
6	+	(+)	+
7	+	0	+
8	+	0	+

From these data we see that *Polycirrus caliendrum* (an alternate host of this commensal, according to Orton) elicits as strong responses from a population of the commensals taken from *Amphitrite gracilis* as the host, and indeed stronger responses than do other members of the host genus, *A. edwardsi* and *johnstoni*. The significance of this strong response to *Polycirrus* is discussed in a further paragraph.

Unfortunately, the author was unable to find a population of *Harmothoë spinifera* commensal with *Polycirrus* in order to make the logical cross-experiments. It would appear that Orton's 'Polynoid B' with *Polycirrus* was *Harmothoë spinifera*, for Mr G. M. Spooner has recently identified as *spinifera* a polynoid with *Polycirrus* taken in dredgings from Plymouth Sound.

HARMOTHOË LUNULATA (DELLE CHIAJE)

This form presents the most fascinating problem of all the polynoid commensals in European waters. It appears to have a number of variations that have been described under different names (Fauvel, 1923); it may be free-living or commensal. Table I, based on what appear to be well-established identifications, lists the hosts of this interesting species, with varietal names ignored. As can be seen, *H. lunulata* has been taken in commensalism with a limited number of species from four distantly related phyla, a situation

TABLE I. HOSTS OF *HARMOTHOË LUNULATA* (DELLE CHIAJE)

CHAETOPODA	
Terebellidae	*Amphitrite edwardsi* Quatre. (Orton*; G.M.S., D.P.W.†)
	A. johnstoni Malmgren (Orton*; G.M.S., D.P.W., D.D.†)
	Polycirrus aurantiacus Grube (McIntosh, 1900; Fauvel, 1923; Orton*)
	Lanice conchilega Pallas (Fauvel, 1923)
Arenicolidae	*Arenicola marina* L. (Fauvel, 1923; G.M.S., D.P.W.†)
Eunicidae	*Marphysa sanguinea* Montagu (Marine Biological Association, 1931; Orton* and McIntosh, 1900: *H. marphysae*?)
GEPHYREA	
Sipunculidae	*Phascolosoma vulgare* Blainville (Orton*; G.M.S., D.P.W.†)
	P. elongatum Keferstein (Orton*)
OPHIUROIDEA	
Amphiuridae	*Acrocnida brachiata* Montagu (Orton*; G.M.S., D.P.W., D.D.†)
HOLOTHUROIDEA	
Synaptidae	*Leptosynapta inhaerens* O. F. Müller (Orton*; G.M.S., D.P.W.†)
	Labidoplax digitata Montagu (Orton*; D.P.W.†)
ASTEROIDEA	
Astropectinidae	*Astropecten irregularis* Pennant (McIntosh, 1874‡)
HEMICHORDATA	
Ptychoderidae	*Ptychodera minuta* [?] (Fauvel, 1923)

* From the collecting notes of the late Prof. J. H. Orton, F.R.S., in the possession of Dr D. P. Wilson at the Plymouth Laboratory.

† Records from the Salcombe and Yealm Estuaries; identification by G. M. Spooner and D. P. Wilson.

‡ A single record, but apparently well-established. McIntosh records its collection by Carrington at Southport *along with Acholoë* (!) from the ambulacral groove.

which somewhat resembles that of the commensals *Arctonoë pulchra* and *vittata* of the Pacific Coast of America (Davenport, 1950).

The difficulty of collecting enough commensals and hosts and of keeping all the animals alive at the same time has unfortunately allowed only the briefest experiments of the type conducted above. It is hoped that ultimately long enough series may be collected of the varieties of this species to permit a careful comparison of each population and its behaviour. However, with the co-operation of the 1953 Easter Classes it was possible to make preliminary investigations of the responses of three different populations of the commensal. At Salcombe, when a large group are digging, a reasonable number of *Harmothoë lunulata* may be collected with the ophiuroid *Acrocnida brachiata* (Montagu). Polynoids commensal with the holothurian *Leptosynapta inhaerens* (O. F. Müller) are not common but may occasionally be found. Likewise at the Yealm estuary *Harmothoë lunulata* is an occasional commensal with the terebellid *Amphitrite johnstoni*, whose more usual partner is *Gattyana cirrosa*.

The data that follow present the host responses of as large a number of these three commensal populations as could be collected by the classes, staff and author in two sets of trips to Salcombe and the Yealm during extreme spring

<table>
<tr><th>Worm</th><th><i>Leptosynapta inhaer.</i></th><th><i>Amphitrite johnstoni</i> tentacle</th><th><i>A. edwardsi</i> tentacle</th><th><i>Polycirrus</i> (sp.?)</th><th><i>Arenicola marina</i></th><th><i>Lanice conchil.</i></th><th><i>Phascolosoma elong.</i></th><th><i>Acrocnida brachiata</i></th></tr>
<tr><td colspan="9">EXPERIMENT NO. 1. COMMENSALS (5–12 MM. LONG) WITH <i>ACROCNIDA</i></td></tr>
<tr><td>1</td><td>o</td><td>o</td><td>—</td><td>—</td><td>—</td><td>—</td><td>—</td><td>(+)</td></tr>
<tr><td>2</td><td>+</td><td>o</td><td>—</td><td>—</td><td>—</td><td>—</td><td>—</td><td>+</td></tr>
<tr><td>3</td><td>+</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td></tr>
<tr><td>4</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>(+)</td></tr>
<tr><td>5</td><td>(+)</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>+</td></tr>
<tr><td>6</td><td>(+)</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>+</td></tr>
<tr><td>7</td><td>(+)</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>+</td></tr>
<tr><td>8</td><td>+</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>+</td></tr>
<tr><td>9</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td></tr>
<tr><td>10</td><td>+</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>+</td></tr>
<tr><td>11</td><td>+</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>+</td></tr>
<tr><td>12</td><td>+</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>+</td></tr>
<tr><td colspan="9">EXPERIMENT NO. 2. COMMENSALS (29–31 MM. LONG) WITH <i>LEPTOSYNAPTA</i></td></tr>
<tr><td>1*</td><td>+</td><td>o</td><td>—</td><td>—</td><td>—</td><td>—</td><td>—</td><td>o</td></tr>
<tr><td>2</td><td>+</td><td>o</td><td>o</td><td>(+)</td><td>o</td><td>o</td><td>o</td><td>o</td></tr>
<tr><td>3</td><td>+</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td></tr>
<tr><td colspan="9">EXPERIMENT NO. 3. COMMENSALS (16–32 MM. LONG) with <i>AMPHITRITE JOHNSTONI</i></td></tr>
<tr><td>1*</td><td>o</td><td>+</td><td>—</td><td>—</td><td>—</td><td>—</td><td>—</td><td>o</td></tr>
<tr><td>2</td><td>+</td><td>+</td><td>o</td><td>(+)</td><td>(+)</td><td>o</td><td>o</td><td>o</td></tr>
<tr><td>3</td><td>o</td><td>+</td><td>o</td><td>o</td><td>(+)</td><td>(+)</td><td>+</td><td>o</td></tr>
<tr><td>4</td><td>(+)</td><td>o</td><td>o</td><td>(+)</td><td>+</td><td>o</td><td>(+)</td><td>o</td></tr>
<tr><td>5</td><td>o</td><td>+</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td></tr>
<tr><td>6</td><td>(+)</td><td>+</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td><td>o</td></tr>
</table>

* First sample collected; untested alternate hosts not available.

tides. The collection and maintenance of the commensals and all the actual and reported hosts tested could quite obviously only have been accomplished with the help of the classes and of Mr G. M. Spooner and Mr P. G. Corbin, to whom the author wishes to express his thanks.

How did the commensals behave? Each population was tested against the actual and reported hosts available at the time (see Exps. 1–3).

If we examine the data of Exp. no. 1, an interesting fact emerges. It can be seen that *one* alternate host of the species, *Leptosynapta inhaerens*, has almost as strong an attraction for the commensals from *Acrocnida* as their own host. None of the other alternate hosts of the species do. However, if we examine the data of Exp. no. 2, keeping in mind that the sample of commensals tested is hardly large enough to allow any definite conclusions, we see that the three *lunulata* from *Leptosynapta* did not respond in return to *Acrocnida*, nor did they respond to any of the other alternate hosts of the species. The significance of these data will be discussed in a further paragraph.

The data from Exp. no. 3 are conflicting. These large active commensals from *Amphitrite johnstoni* are as difficult to work with as *Gattyana cirrosa*, for they are strongly affected by visual and tactile stimuli. They exhibit a powerful response to surfaces and some of their delayed and sluggish responses to alternate hosts (*Polycirrus*, *Arenicola*, *Phascolosoma*) may have been the result of other factors than chemical recognition. It is clear that they respond with the usual intensity to their own host, but a larger series must be assembled and perhaps a better experimental technique developed before an adequate comparative study can be made of their behaviour in relation to the various alternate hosts of the species.

DISCUSSION

Studies on the specificity of responses have now been made in several genera of polynoids commensal with echinoderms and with other annelids. In the most extensive series of experiments (Davenport, 1953) conducted on echinoderm commensals (*Astropecten-Acholoë*), it has been shown that the polynoids respond to a number of starfish genera belonging to two taxonomic orders, which gives indication of some biochemical affinity among these rather distantly related starfish, at least as concerns the production of attractant. Perhaps here there is either a genetically determined (innate) or individually conditioned response to a substance which is produced by a number of different starfish, some of which are not the host of the polynoid. But in this type of association the specificity of response may not depend solely on chemical recognition of single factors acting as a bond between commensals. The possibility must be kept in mind that recognition-responses may be elicited by a particular pattern or sum of stimuli, part of which may not be chemical. Annelids are relatively highly organized and it has certainly been demonstrated in many organisms with a fairly high level of nervous organization that recognition by chemical means alone may depend upon stimulation by a pattern or combination of substances. Responses of full intensity by commensals may, then, conceivably depend upon just the right combination of stimuli, a pattern produced only by the host. Any alteration of the pattern may dampen or abolish responses, so much so as to prevent the 'colonization' of species to which a delayed response may be given. Here, perhaps, the bond may simply not be strong enough to maintain the 'colonization'. In addition, many other ecological factors may prevent it.

However, the specificity of response of polynoids commensal with other annelids seems somewhat more strictly limited than that of the single echinoderm-annelid partnership the specificity of which has been investigated. In *Polynoë scolopendrina*, *Lepidasthenia argus*, and *Harmothoë spinifera*, responses, with one interesting exception, appear to be limited to members of the taxonomic family (Terebellidae) of the host from which the commensal population was taken. Responses to host species are far stronger than any that may be demonstrated to non-host members of the same genus or related genera. The tests with bolting-silk strongly suggest that the stimulus of primary importance in host recognition is chemical. It would appear, then, that these rather specific polynoid-terebellid associations may have in part evolved as a result of the development of innate or individually conditioned sensitivities to rather specific biochemical substances produced by their hosts (and perhaps at times in reduced quantity or in modified state by their host's relatives). That the attractants are relatively specific and differ to a recognizable extent from each other is indicated by the fact that *Polynoë scolopendrina*, *Lepidasthenia argus*, and *Harmothoë spinifera* will give constant responses of full intensity to their own hosts but not to each other's.

The tests in which *Polynoë scolopendrina* clearly exhibited a strong response to the eunicid *Lysidice ninetta* are of considerable interest. *Lysidice* may harbour this *Polynoë* (Channel Islands—McIntosh, 1900), but the association has not been recorded in Plymouth waters, where *Lysidice* is as relatively available as the normal host. At any rate the experimental population of *Polynoë* was taken in association with the terebellid *Polymnia nebulosa*, the usual host of the scale-worm at Plymouth. These commensals demonstrate no response whatever to members of three genera of terebellids which are certainly much more closely related to the host *Polymnia* than to the eunicid *Lysidice*.

Equally suggestive were the experiments in which *Harmothoë spinifera* taken with *Amphitrite gracilis* appears to respond more strongly to the alternate host *Polycirrus caliendrum* than to other species of *Amphitrite*.

Here for the first time evidence is presented that in single species which may occur commensally with unrelated hosts, the specificities, at least in some cases, may depend upon the production of similar attractants by the unrelated hosts. Yet the experiments with the populations of *Harmothoë lunulata* are not quite in accord with this theory. The fact that *lunulata* taken from the brittle-star *Acrocnida* responded equally strongly to both *Acrocnida* and the alternate host holothurian *Leptosynapta inhaerens* seems in line with it, but were the attractants of these two host animals for their own commensal population in fact the same, then the population taken from *Leptosynapta* should have given an indication of a response to *Acrocnida*. Perhaps the *lunulata* from *Acrocnida* are adapted to a substance common to both the brittle-star and the holothurian, while those from *Leptosynapta* will respond to a different substance released by their host alone. Certainly the failure of the three commensal populations of *Harmothoë lunulata* to respond to the great majority of the alternate hosts of the species would argue that each of the three populations is adapted to respond to a substance not released by these alternate hosts.

Nevertheless, it would seem that if responses are the result of individual conditioning and are not innate, the attractants of such hosts as *Polymnia nebulosa* and *Lysidice ninetta* and of *Amphitrite gracilis* and *Polycirrus caliendrum* must be the same or very nearly so. On the other hand, if the response is innate and not individually conditioned (which seems to the author most likely, in view of the specificity and obvious long establishment of these associations), then the attractants may be the same or they may be different, under which latter circumstance one would have to admit the existence within a single commensal population of innate and highly precise responses to more than one attractant substance.

It is entirely possible that such multi-specific genetically determined responses may be important in governing the specificity of single commensal species that inhabit unrelated hosts. This again can only be determined by breeding experiments and the use of much larger samples of experimental material for behaviour studies than one has ordinarily available from restricted populations uncovered only rarely at extreme low tides. Certainly a deeper understanding of the mechanism of specificity of marine commensal associations can be obtained by a continued investigation of the behaviour of such species as *Harmothoë lunulata* existing as it does with such a diversity of hosts. If, as has been suspected (Wilson, 1951), the forms of *lunulata* which in ascending size respectively inhabit *Acrocnida*, *Leptosynapta* and *Amphitrite johnstoni* are nothing but different age-groups and not physiological races which can be separated on the basis of host-specificity, then one would have to hypothesize a different response-specificity for each age-group, determined perhaps by the state of development of the commensal. The behaviour studies on *Harmothoë lunulata* described above cast little light on this facet of the problem.

In an estuary such as Salcombe this *Harmothoë* occurs in association with so many animals that one may be tempted to conclude that it will occupy 'almost any convenient hole'. It is unquestionably true that the host specificity of marine commensals is 'superimposed' upon a number of other basic ecological requirements. Laing (1937) has ably demonstrated in the insects *Alysia* and *Mormoniella* that these parasites 'are attracted to an environment likely to contain their hosts by qualities of the environment itself, independent of the presence of hosts'. Whether this is also so in commensal polynoids, in which the early stages may have a long planktonic life, can only be determined by settlement and metamorphosis studies. At any rate, adequate data on the response-specificity of commensal populations must be assembled before such factors as the physical nature of the host's tube and the currents in it may be considered of primary importance in determining the specificity of the associations.

As a result of preliminary experiments with terebellid commensals (Davenport, 1953), the author stated that his observations indicated 'that a chemotaxis to the host may be of relatively minor importance in governing the behaviour of these commensals'. Clearly, this conclusion is no longer tenable; the observation of Orton & Smith (1935) that 'there is a tropic response on the part of the polynoid, causing it to enter an *Amphitrite* burrow whenever possible' was, although unsupported by data, correct.

Finally, all the studies so far conducted on echinoderm-annelid and annelid-annelid commensal partnerships indicate that normally commensals are restricted to their hosts by a positive chemotaxis to an unstable or closely-bound substance or pattern of substances. The adaptive significance of an unstable or closely bound attractant is clear; the function of the attractant could hardly be served if it were released in quantity and failed to break down or be rapidly destroyed or diluted in the environment of the partners.

SUMMARY

The host-response of the polynoid *Polynoë scolopendrina* Savigny, commensal with the terebellid *Polymnia nebulosa* (Montagu), is relatively specific within the family Terebellidae.

Evidence is presented that an unstable or closely bound attractant is present on the outside of the host and perhaps in tube material, but apparently absent in the mucus secreted by the host. Efforts to demonstrate its presence in ground-up whole host resulted in failure.

The polynoid *Lepidasthenia argus* Hodgson demonstrates a similar specificity of response to its host *Amphitrite edwardsi* Quatrefages.

The polynoid *Harmothoë spinifera* Ehlers, commensal with *Amphitrite gracilis* Grube, responds to the alternate host *Polycirrus caliendrum* Claparède as strongly as to its own host, and more strongly than to other species of *Amphitrite*. Likewise, *Polynoë scolopendrina*, commensal with *Polymnia*, responds with greater intensity to the alternate host, the eunicid *Lysidice*

ninetta Audouin and Milne-Edwards, than to any non-host terebellid. This behaviour may be interpreted as evidence that the specificity of at least some single commensal species for hosts which are not closely related to each other may depend upon the production of similar attractants by these hosts.

The responses of three commensal populations of *Harmothoë lunulata* (Delle Chiaje), commensal respectively with the ophiuroid *Acrocnida brachiata* (Montagu), the holothurian *Leptosynapta inhaerens* (O. F. Müller), and the terebellid *Amphitrite johnstoni* Malmgren were tested against seven alternate hosts. Each gave maximum response to its own host. However, the population from *Acrocnida* responded as strongly to *Leptosynapta* as to its own host, while giving no responses at all to any of the other five.

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J. Mar. biol. Ass. U.K. (1953) 32, 289–296
Printed in Great Britain

MOULTING HORMONES IN *LEANDER* (CRUSTACEA DECAPODA)

By D. B. Carlisle
The Plymouth Laboratory

(Text-fig. 1)

It is now well known that the initiation of the premoult in certain species of crabs and in the Astacidae is under the control of a moult-inhibiting hormone. Megušar (1912) observed that the removal of the eyes in *Astacus* led to an earlier moult. This was confirmed in the related *Cambarus* by Brown & Cunningham (1939). In the meantime, the same phenomenon had been observed in crabs of the genera *Uca* (Abramowitz & Abramowitz, 1938, 1940) and *Eriocheir* (Hanström, 1939). Kleinholz & Bourquin (1941) confirmed the finding for *Uca*, and Smith (1940) for *Cambarus*. Smith showed that this was not a result of injury alone. Brown & Cunningham (1939) followed eyestalk removal with implants of sinus glands and found that this prevented the early moult. They suggested that the sinus gland secreted a moult-inhibiting hormone whose absence allowed moulting to proceed. Kleinholz & Bourquin (1941) doubted this conclusion, but the work of Scudamore (1942, 1947) and Kyer (1942) on *Cambarus* went far to substantiate it. By 1947 it was generally accepted that the eyestalk secreted a moult-inhibiting hormone, and most workers believed that this originated in the sinus gland.

In 1951 a number of workers independently published papers which, while substantiating the hypothesis of a moult-inhibiting hormone, threw doubt on the suggestion that it originated in the sinus gland. Bliss (1951) working on *Gecarcinus*, Passano (1951 *a*, *b*) on *Sesarma*, Frost, Saloum & Kleinholz (1951) and Havel & Kleinholz (1951) on *Astacus*, and Welsh (1951) on *Gecarcinus*, all found evidence which suggested that the true source of the moult-inhibiting hormone was the X-organ and that the sinus gland was merely a store. The story has been more fully worked out by Passano (1952 *a*, *b*) and by Bliss & Welsh (1952). Briefly these authors believe that the moult-inhibiting hormone is formed in neuro-secretory cells of the X-organ, transported within the fibres of the sinus gland tract to the sinus gland, which consists merely of enlarged nerve endings, and there stored until finally released into the blood stream. Carlisle (1954 *c*) has produced evidence that the same may be true of the ovarian-inhibiting hormone in the Mediterranean prawn *Lysmata*. Now the X-organ of most crabs and crayfish is specialized in that the two parts are united into a single organ, while in stomatopods, prawns and lobsters, hermit crabs and *Dromia* they are separated (Carlisle & Passano, 1953). Moreover, moulting in the species which have been investigated in the studies mentioned above seems to be characterized by its occurrence at specific seasons of the year—moulting seasons. *Cambarus*, for instance, moults in spring and autumn. Prawns, lobsters and some crabs, on the other hand, moult all the year round, and *a priori* we may suspect that moulting may never be directly inhibited in them. Travis (1951), indeed, failed to find any evidence of a moult-inhibiting hormone in the spiny rock lobster *Panulirus*, which has no definite moulting season and an intermoult period of 80 days under the condition of her experiments. The experiments reported here represent a failure to find any evidence for its occurrence in *Leander* (=*Palaemon*) *serratus*.

EXPERIMENTAL DATA AND CONCLUSIONS

Below 11° C. the rate of moulting of *Leander* is negligible, but it rises sharply above this temperature. All the observations recorded here were made at a temperature of $13{\cdot}5 \pm 1°$ C. The average intermoult period at this temperature was estimated by keeping 150 female prawns, between 55 and 70 mm. long (measured from the tip of the rostrum to the tip of the telson), through two or three successive moults, and taking the arithmetical mean of the individual intermoult periods. The prawns were kept singly in Breffit jars (4 lb. rock jars) in 1 l. of water each. They were fed twice weekly on squid flesh and the water was changed 3 hr. after feeding. Under these conditions the mean intermoult period was $34{\cdot}62 \pm 3{\cdot}19$ days.

Two hundred female prawns of the same size range were then selected, rejecting all which had recently moulted and were still soft. These were divided into two equal batches at random, using Fisher & Yates's (1943) table of random numbers for the purpose. Both eyestalks were removed from the individuals of one batch by electrocautery. The eyestalks were cut through at the base, at the level of the arthrodial membrane, using a red-hot platinum cautery needle. It was found that if the eyestalks were cut with scissors or with a knife and then cauterized the death-rate was much higher than if the whole operation was performed by cautery. This may be correlated perhaps with the complete absence of blood loss after direct cautero-ablation. These hundred operated animals were placed in a tank with the other hundred unoperated animals and left for 7 days; during this period they were fed twice *ad lib.* with squid flesh. Fifty from each group were then isolated in Breffit jars, one per jar, in 1 l. of sea water each. From then on until every animal in one group had either died or moulted the numbers which moulted and of those which died without first moulting were recorded each day in each group. The animals were fed twice weekly on squid flesh and the water changed 3 hr. after feeding. On the twenty-sixth day of this treatment the last animal, in the unoperated group, which had not already moulted, died. By that time only three animals in the operated group had neither died nor moulted. The date on which each animal moulted was recorded on its jar, and after the twenty-sixth day those which were still alive, having already moulted, were continued under the same treatment until they either moulted again or died. In this way it was possible to determine empirically the intermoult period of some of the animals under these conditions. The figures are given in Table I. The average intermoult period calculated from these figures is 33·78 days for the unoperated controls and 39·40 for the operated animals. The difference is not significant ($P=0{\cdot}1$).

TABLE I

	Operated	Unoperated controls
	31	27
	36	30
	40	30
	44	31
	46	34
	—	35
	—	36
	—	40
	—	41
Mean	*39·40*	*33·78*

Intermoult periods in days of those animals which survived through two moults.

The numbers which died before moulting and of those which moulted during the earlier part of the experiment are given in Table II and the moult rate is illustrated graphically in Fig. 1. From these data we may obtain, by a calculation from the moult rate, an estimate of the average intermoult period in the animals of the two groups; in the unoperated controls this figure is 35·46 days and in the operated group 47·30 days. Statistical analysis of the data indicates that the difference in moult rate is not significant ($P=0{\cdot}5$). The method of analysis adopted was that used and described by Carlisle & Dohrn (1953).

There is no evidence from either of these tests in the one experiment that removal of the eyestalks results in an increased rate of moulting, a shorter intermoult period or earlier moulting; rather, in fact, the opposite. But have we used enough animals for this to be a fair test?—and has the experiment continued for long enough? The average intermoult period for unoperated animals at the temperature and under the conditions of the experiment has been calculated three times in two different ways; there is adequate agreement between the figures. The highest value of the three is 35·46 days. Now the first part of the experiment was concluded 7+26 days after the eyestalk ablation was performed—33 days afterwards. The removal of the eyestalks in crabs and crayfish initiates the premoult, or proecdysis. If the removal of the eyestalks in *Leander* in these experiments had initiated proecdysis, this phase of the moult cycle must be longer than 33 days as an average, for the moulting which terminates the premoult had not occurred *en masse* at the end of this period. But the average intermoult period, including metecdysis and diecdysis as well as proecdysis, is only 35·46 days (taking the highest estimate—33·78 days if we take the lowest). This then only leaves 2·46 days (or 0·78) for the other stages of the intermoult besides the premoult, which is obviously out of the question. In other words, the first part of the experiment has continued long enough to test whether removal of eyestalks initiates proecdysis. Even more is this true for the second part, the continuation of the experiment.

TABLE II

	Operated		Unoperated controls	
Day	Dead	Moults	Dead	Moults
1	1	0	0	1
2	2	1	2	0
3	1	1	0	2
4	1	0	0	0
5	0	1	1	0
6	2	1	11	1
7	0	0	3	0
8	0	0	0	0
9	3	3	0	0
10	0	0	0	2
11	0	4	0	1
12	0	1	0	3
13	0	2	0	2
14	2	1	1	1
15	0	1	0	0
16	4	0	0	2
17	4	0	0	0
18	3	0	0	0
19	1	0	0	1
20	1	2	1	1
21	1	1	1	1
22	0	0	0	4
23	1	0	2	4
24	0	0	1	0
25	0	1	0	0
26	0	0	1	0
Totals	27	20	24	26

The numbers of animals which died without first moulting and of those which moulted on each day of the experiment.

From the data in the second part of the experiment we may calculate the probability that the mean intermoult period in the operated group is actually lower than that in the unoperated group, even though in our sample the reverse is true. The probability of this is about 5%, which is the usually accepted level of significance. This suggests that the number of observations made in the second part of the experiment is just adequate. Since more observations were made in the first part of the experiment the numbers were evidently adequate there also, and, moreover, the difference between the mean intermoult periods in the two groups is more pronounced in this first part.

It seems clear that whatever effect the removal of the eyestalks has had on the moulting of *Leander* under these conditions it has not produced an abbreviated intermoult period, an earlier premoult or a higher moult rate. In fact there is an indication that the converse is true. Every experiment of this type that I have undertaken on *L. serratus* (=*Palaemon serratus*) or *L. squilla* (=*P. elegans*) has given a result similar to that found here—a lower moult rate and a longer intermoult period in the operated animals than in the unoperated controls, but with a difference that was never significant. These experiments took the form described above, but at varying temperatures and for varying lengths of time. None of them showed a significant difference in the moult rate. But when a compound probability is calculated for the difference in moult rate and intermoult period in the two groups (due attention being paid to the sign of the difference) over the six experiments of this type which have been performed with these two species, the value of this statistic is less than 0·01. The average intermoult period was, as mentioned above, longer in the operated than in the unoperated groups. That is to say, far from demonstrating that removal of eyestalks leads to an increased moult rate and an abbreviated intermoult period, these experiments when taken together show just the opposite effect and the degree of the effect is significant. If we argue along the same lines as the workers who discovered the moult-inhibiting hormone in crabs and crayfish we shall conclude that in removing the eye-

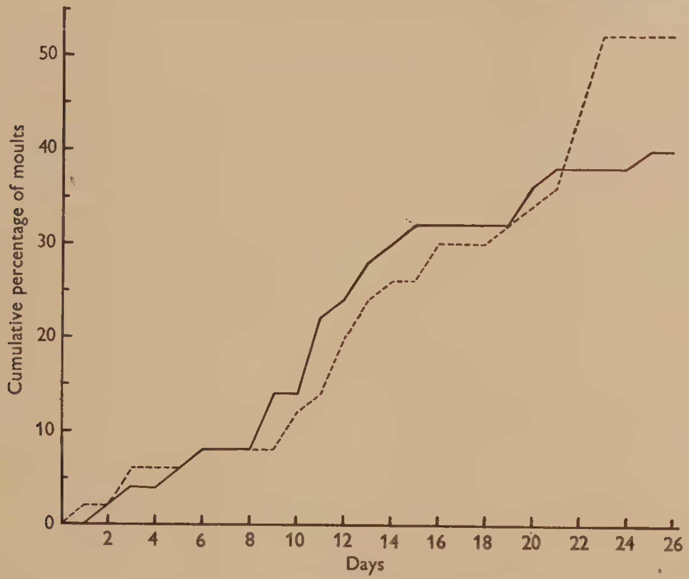

Fig. 1. Graph of the cumulative percentage of moults in the animals whose eyestalks had been removed (——) and in the unoperated controls (- - -). The number of days is measured from the date on which the animals were isolated, not from the date of the operation, which took place 7 days previously.

stalks we are removing some organ which secretes a moult-accelerating hormone. The presence of such a hormone has already been demonstrated in the Mediterranean prawn *Lysmata* (Carlisle & Dohrn, 1953; Carlisle, 1954*a*) and it has been shown that it occurs in both parts of the X-organ (Carlisle, 1954*b*). Carlisle & Dohrn (1953) also showed that this hormone was present in extracts of eyestalks of *Palaemon* (=*Leander*) spp. when tested on *Lysmata*. Evidently, then, in removing the eyestalks of *Leander* we have reduced the amount of moult-accelerating hormone available to the animal (we have not removed the total supply, for it is produced in the thoracic and cerebral ganglia as well, see Carlisle, 1954*b*) and hence we have increased the length of time which the prawn needs to prepare for moulting.

Discussion

The evidence regarding the distribution of the moult-inhibiting hormone of the eyestalk no longer permits us to say that it is universal in the decapod Crustacea. Certainly it is found in most crabs that have been investigated and among the Macrura in the Astacidae. It has not been found in *Panulirus* in Bermuda (Travis, 1951), nor has it been found in *Leander* at Plymouth. Neither *Panulirus* nor *Leander* has a definite moulting season, whereas many crabs and the Astacidae do have a definite season. The coast of North America has more species of large Crustacea which have a seasonal moult than does the coast of Britain. Possibly this is why American workers have found evidence for the moult-inhibiting hormone in all the species that they have investigated. The larger proportion of seasonal moulters on the North American coasts may perhaps be correlated with the Labrador Current of cold water which streams down from the Arctic Sea, whereas in Britain the sea is warmer from the North Atlantic Current arriving from tropical waters. Clearly, in polar waters the seasons are more pronounced than in tropical waters and it would be a hardy crab which would moult other than in the summer in the Arctic Sea. The only decapod in Plymouth waters which has a clearly circumscribed moulting season, so far as I can ascertain, is *Maia squinado*, a northern form, which at Plymouth is towards the southern end of its range. It is possible, then, that the moult-inhibiting hormone is associated with seasonal moulting and with a cold water distribution, although it may well be present in other forms also which had perhaps a cold water ancestry.

Summary

Removal of the eyestalks in *Leander serratus* does not result in an earlier moult, a shorter intermoult period, or a higher moult rate. There is no evidence of an eyestalk moult-inhibiting hormone in this species. The evidence points to the existence of an eyestalk moult-accelerating hormone. The possible correlation of the presence of the moult-inhibiting hormone and seasonal moulting is discussed.

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J. Mar. biol. Ass. U.K. (1953) 32, 297–304
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SIZE VARIATIONS IN THE CYPRIDS OF SOME COMMON BARNACLES

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(Text-figs. 1–4)

In descriptive works on cirripede larvae some of the dimensions of the various developmental stages are often quoted as an aid to identification, and data have already been given in this form for *Balanus balanoides*, *B. crenatus* and *Verruca stroemia* (Pyefinch, 1948*a*; Bassindale, 1936; Runnström, 1925). There appear, however, to be no data on variations in size. The present account gives some observations on local variations, a discussion of which is a necessary preliminary to a study of regional variations and their significance.

The Collection and Examination of the Material

A number of different sampling methods have been used but most of the material has been collected by means of a Hardy Plankton Indicator (large model). The indicator was towed in the standard manner for a fixed distance (about 1 mile), the material being also collected for an investigation of annual changes in the barnacle population of this region. Such a method has the disadvantage that only one level is sampled, but other work has shown that the distribution of cyprids is fairly uniform between 0–20 metres. After completion of the tow the indicator was taken inboard, the plankton carefully washed off the silk into a bottle, and neutral formalin (5%) added. The whole sample was counted and measured, the measurements being made by means of a calibrated eye-piece micrometer. These hauls were taken regularly throughout the spring and were also supplemented by net hauls: later in the year a number of net hauls were made, and opportunity was also taken of using material collected by Mr V. Bainbridge during the course of his own work.

Attention has been largely confined to the cyprid stage, since this can be more easily and more accurately measured, but some nauplii have been measured. Results are presented for three species, *Balanus balanoides*, *B. crenatus* and *Verruca stroemia*, collected over the varying periods. The total length and the greatest width were measured with the cyprid lying on its side. Attention will primarily be concentrated on the lengths; but similar deductions can be made using the width measurements. In the stage VI total length and greatest width of the carapace were measured with the animal lying on its ventral surface. The scale of magnification was 50 eye-piece units = 1·02 mm., but it will be more convenient in the discussion of the results to refer to the arbitrary units. All the material was preserved in neutral formalin (5 %), and strictly speaking the measurements refer only to animals preserved in this way.

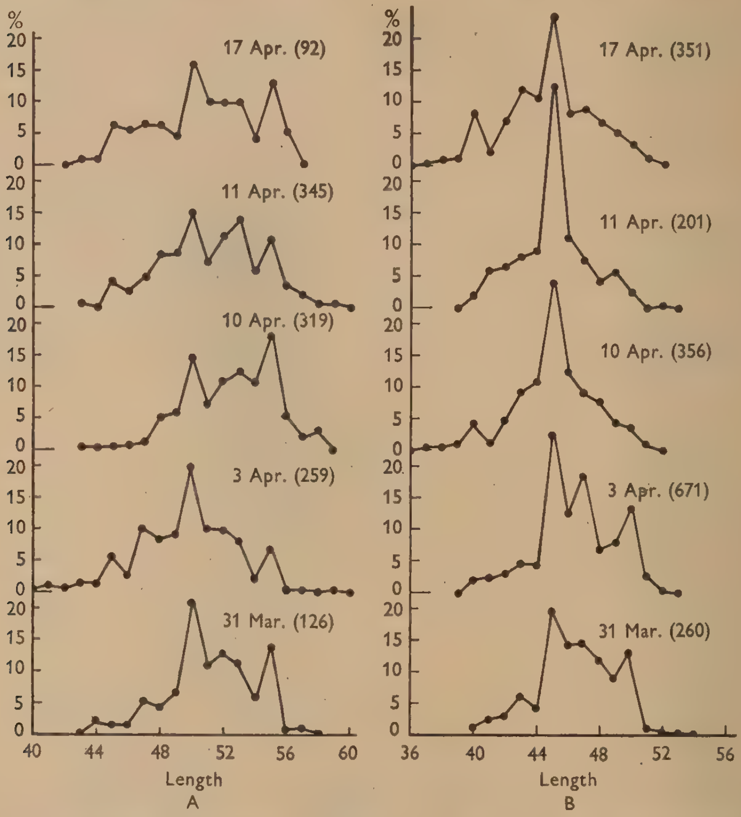

Fig. 1. Size-frequency distribution of *Balanus* sp. cyprids in a series of hauls through the spring. Length in eye-piece units. Dates and numbers in collections are given. A, *B. balanoides*; B, *B. crenatus*.

THE SPRING OUTBURST OF *BALANUS BALANOIDES* AND *BALANUS CRENATUS*

The results for the cyprids of these two species are shown in Fig. 1, which gives the size-frequency curves together with the numbers of the animals measured, and the dates of collection. Even if the minor peaks are neglected it is clear that with both species these size-frequency curves exhibit a number of modes, which are to be presumed to correspond to a number of populations of the cypris stage each with its own mean size.

In *B. balanoides* the mode which is found at 50 units is present and well marked throughout the whole of the sampling period. A mode at 55 units is quite well marked in some of the samples, and there is a suggestion of other modes at intermediate points. In the earliest sample the 50-unit mode represents some 35 % of the total frequency and is stronger than in the later samples. There is a tendency for the second mode at 55 units to be greater in strength later in the season. A study of the cumulative frequency curves supports the above deductions, but in view of the considerable overlap in the sizes they are not easy to analyse with certainty. It seems reasonable to suggest, however, that there are at any rate two distinct populations and that these are present throughout the season.

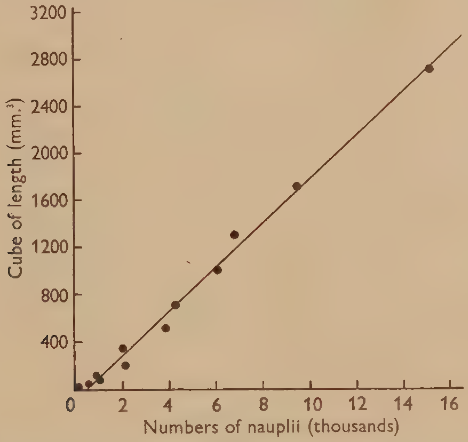

Fig. 2. Larval output and size of adults in *B. balanoides*. The total number of nauplii is plotted against the cube of the adult length. Grouped values are used.

In *B. crenatus* two modes are also present in the earlier samples, both quite strong, but as the season progresses the population giving rise to the higher modes disappears. This can only mean that one of the populations (larger size) concerned is produced only at the beginning of the season, whilst the other population is produced continuously throughout the season.

The Environment and Reproduction

Moore (1935) has shown that the number of larvae produced by a barnacle is a function of its size, a fact confirmed in this investigation. Fig. 2 shows a plot of the mean nauplii content for a series of size-groups against the cube of the length, which is approximately a linear function of the volume. It has also been shown (Moore, 1934; Barnes & Powell, 1953) that the size attained by the adult barnacle at any given time is a function of a whole complex of conditions. However, since the conditions of the environment have such a marked influence on the growth-rate and on the reproductive capacity, it is important to examine whether they also affect the size attained by the nauplii at the time of extrusion. It has been possible to measure the size of ripe nauplii of known age at various levels on the shore. During his work on algal colonization Mr Powell had completely cleared a number of rocky strips of barnacles and other organisms, and a number of 1-year-old barnacles were collected from the upper and lower levels on these strips on which zonation was just developing at the time of the spring outburst. These adults were all ripe and the embryos were teased out under water, when every time some nauplii escaped from the egg cases. The total length of a number of nauplii was measured whilst in the egg cases; the values obtained were as follows (50 units = 0·423 mm.):

	Mean size	
	Barnacles (mm.)	Nauplii (units)
Upper level (strip I)	6·67	34·65
Upper level (strip II)	6·72	34·73
Lower level	3·50	33·61

In these collections the content of nauplii and size were again correlated, and the above results also indicate that the conditions which have affected the growth of the adults and their larval output have, in addition, affected the size attained by the nauplii. This is confirmed by samples (of unknown age-group) taken from the pier; at the upper levels the mean naupliar size was 34·94 units and at a very low level it was 32·26 units.

THE ORIGIN OF THE CYPRID POPULATIONS

Cyprid populations may come to be different in size from more than one possible cause. The populations may represent different races, that is, be genotypic in origin. In view of the extensive mixing of the cyprids in the surface waters during the planktonic phase and their ultimate distribution over the whole intertidal zone in *B. balanoides*, together with the necessity for cross-fertilization, it seems unlikely that distinct races of adults giving rise to populations of different-sized cyprids would be developed. Further, when the frequency curve for the length/breadth ratio is plotted it is almost unimodal, in marked contrast to those for the length or breadth, suggesting that the populations are only different in size and not in shape (Fig. 3). Again, the populations may exhibit growth differences as a result of the effect of the environment during the planktonic development. The persistence of the separate populations in *B. balanoides* throughout the spawning season and the existence of differences in the nauplii within the ripe adults from different levels tends to negate this suggestion.

A more plausible suggestion is that in *B. balanoides* the modes represent the product of the different zones of the shore, for, in view of the above results, if the nutritive conditions are sufficiently well defined in the different zones there will be a tendency to produce a series of different-sized nauplii giving rise to different-sized cyprids which correspond to these levels with, of course, overlapping. Evidently, with *B. balanoides*, the upper and lower zones of the shore are sufficiently different to give this result, the environment and growth conditions at the two levels being sufficiently distinct. The growth-rate of *B. crenatus* also has been shown to vary according to the habitat, being far greater under the more favourable conditions (Barnes & Bagenal, 1951; Barnes & Powell, 1953); but since this species is largely sublittoral in this region it is less clear why two distinct populations are produced. Perhaps those adults which just extend into the littoral and near-littoral zone are responsible for one population, whilst the deeper water populations produce the other. If this is so, since the major population of *B. crenatus* adults is certainly sublittoral, the near-littoral group must produce the larger cyprids, which constitute the smaller proportion of the total population (taken over the whole sampling period), and the sublittoral population must continue to spawn over a longer period, for the larger mode tends to disappear in the later collections (see Fig. 1B). It is, however, possible that the populations correspond to the products of the different year-classes of adults.

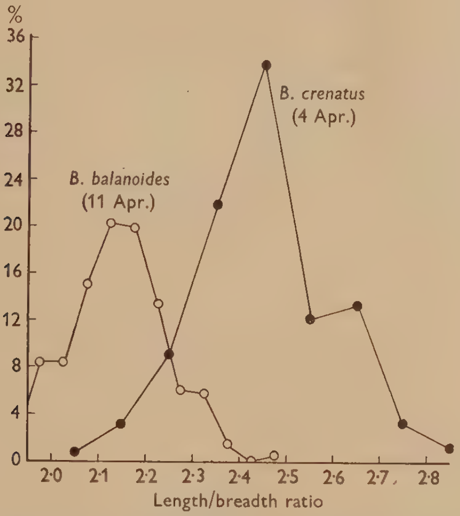

Fig. 3. Size-frequency of length/breadth ratio for *B. balanoides* and *B. crenatus*, for samples taken on 11 April and 4 April respectively. Grouped values are used. Compare with curves in Fig. 1.

THE SIZE DISTRIBUTION OF SWEDISH *BALANUS BALANOIDES*

The tidal rise and fall at Millport is of the order of 10 ft., sufficient to give large changes in environment with distinct algal zonation, and to give rise to at least two populations. On the west coast of Sweden the tidal rise is 1 ft. or less and on the preceding hypothesis only a single-sized population of cyprids might be expected. Through the kindness of Dr Höglund it has been possible to examine some plankton collections taken in late March and early April from the Altane Fjord (58° 45′ N., 11° 15′ E.). The size-frequency curve is given in Fig. 4, and apart from the slight break at 53 units is unimodal with the mode at 56 units. It stands therefore in marked contrast to the type of size-frequency curve (Fig. 1 A) given by the same species from British waters at Millport, and the difference supports the hypothesis given above regarding the effect of zonation.

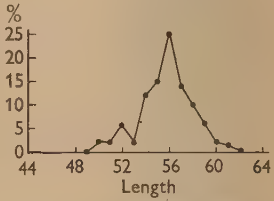

Fig. 4. Size-frequency distribution of Swedish *B. balanoides*. Length in arbitrary units.

CHANGES IN SIZE DURING THE YEAR

At Millport *B. balanoides* breeds only once a year, but *B. crenatus* and *Verruca stroemia*, in addition to a main outburst in the spring, produce several later although much smaller (in numbers) broods (Pyefinch, 1948).

Verruca stroemia

As pointed out by Pyefinch there seems to be a delay in the production of cyprids of this species, and, moreover, cyprids are never caught in quantities comparable with the nauplii, in surface or subsurface collections. However, small collections were obtained in both spring and autumn and have been examined. As far as can be determined from the smaller numbers (62 in each set, pooled samples from several days) the size-frequency curves for both collections are unimodal; and, since this is a genuine sublittoral species of fairly restricted habitat where conditions are uniform, this finding affords additional support for the previous hypothesis with regard to the other species. The mean lengths and their standard errors are given below for the spring and autumn cyprids:

	Mean length	S.E.	Ratio, L/B	S.E.
Spring	30·8	0·163	2·34	0·0196
Autumn	27·70	0·190	2·33	0·0185

Balanus crenatus

Only comparatively few (27) were obtained during the autumn, insufficient for adequate representation of a size-frequency curve. They have therefore been compared with the unimodal spring curves. Since it is the smaller-sized mode that persists, any size decrease during the season will tend to be minimized:

	Mean length	S.E.	Ratio, L/B	S.E.
Spring	46·36	0·117	2·47	0·00695
Autumn	41·93	0·399	2·46	0·0267

In both these species, therefore, which produce more than one brood a year, there is a significant decrease in size in the cyprids of the later broods although, as far as can be judged from the length/breadth ratios, there is no change in shape.

Size variations in successive broods during a year have often been reported for copepods (see, for example, Marshall, 1933, 1949) and both temperature and food are considered to be important factors. In general, larger animals are found when development takes place more slowly at lower temperatures. However, it has often been difficult to reconcile the facts with the actual changes in temperature. It is suggested that the rate of development of the eggs or of the embryos within the parent may be as important a factor as the rate of development during the planktonic stages in determining the size of any given stage. This might explain to some extent the unexplained fluctuations found by Marshall in the sizes of copepod stages, since although water temperatures in general rise during the summer, if size is to some extent determined by the rate of development, temperature fluctuations acting over a much shorter time could be important.

In the case of *B. crenatus*, considered above, development of the spring brood of nauplii takes place during the autumn and winter months, October to March, from eggs fertilized in the autumn. Development of the embryos is therefore slow and occurs during the period when the sea temperatures are low. Development of the autumn brood is more rapid, affected by higher temperatures. The brood may even be produced, according to Pyefinch (1948 *b*), from animals settled in the same spring; in consequence, on the above hypothesis, the nauplii and hence the cyprids so produced, would be expected to be smaller.

Thanks are due to Dr Höglund for providing the Swedish samples, to Mr V. Bainbridge for allowing the cyprids to be used from some of his collections, and to Mr H. T. Powell for providing the barnacles of known ages from his rock strips.

SUMMARY

The sizes, length and breadth, of the cyprids of *Balanus balanoides*, *B. crenatus* and *Verruca stroemia* have been measured on material collected during the spring outburst and in the later part of the year.

The size-frequency curves for *Balanus balanoides* and *B. crenatus* show at least two distinct modes during the spring, equivalent to populations of two

distinct sizes. It is suggested that these correspond to populations developed in different environments. Measurements of the nauplii taken from adults at different points on the shore indicate that the size of the nauplii is dependent to some extent upon the conditions of development, the conditions also affecting growth and total nauplii output.

Swedish samples, in which the parents had developed under more uniform conditions, showed only a single population as regards size.

In *B. crenatus* and *Verruca stroemia*, which produce further broods during the year, the size decreases in these later broods. The rate of development of the eggs and embryos, probably dependent upon temperature, is suggested as the important factor.

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J. Mar. biol. Ass. U.K. (1953) 32, 305–313
Printed in Great Britain

BIOMETRY OF THE COPEPOD *CALANUS FINMARCHICUS* (GUNN.) IN STAGES V AND VI

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(Text-figs. 1, 2)

There is much published information on the size of the several stages and its variation in the copepod *Calanus finmarchicus*. Marshall (1933), in the Clyde area, has found considerable variations in size even between the different generations developed throughout a single season, such variations extending to most of the copepodite stages. In general, the generations developed earliest in the season were the largest: further, the difference in size between the male and female stage VI copepodite was found to be considerable in the spring brood but almost negligible in a summer brood. Differences in size have also been reported with depth (Russell, 1928; Gardiner, 1933), and there are differences in the size of animals from distinct geographical regions. It has also been stated that the relative proportions of metasome and urosome vary. However, while some of these observations relate to the total length of the metasome and urosome taken together, most have been made on the metasome only: there appear to be no measurements of the individual segments and no consideration of the changes of size of the segments from one stage to the next. Further, the question is still open whether any sexual differentiation in size is evident in copepodite stage V.

The Materials and Experimental Methods

One of the difficulties in analysing the size variation in *Calanus* and other copepods arises from the fact that in most collections, even when obtained from a restricted area, there is often a mixture of two populations as a result of some overlap in the generations. Further, there is considerable spread, even in the sizes of individuals of a given stage from a distinct generation and this, together with the overlapping of the generations, has led to the use of overall means or modes when sizes are recorded. Certain features of the results have therefore been lost. Such an overlap of the sizes, although always likely to be present can to some extent be minimized by a choice of the time of collection.

In this work, therefore, all the results have been plotted as cumulative percentages, and in this way the presence of more than one population detected. Further, from the plots the means and standard deviations of the separate populations have been determined graphically.

The animals here referred to are *C. finmarchicus* in the sense given by Rees (1949).

The material used was collected in the Firth of Clyde at a constant depth of 100 m. with a tow-net hauled horizontally. As shown by a preliminary examination at the time of collection, late June, it consisted largely of stage VI, males and females (10·6 and 26·2% respectively), stage V (56·2%), stage IV (6·2%) and only a few of the early stages. It seems clear, from a comparison with the results of Marshall (1933) that this was the first summer generation and that the stage V population was giving rise to the population of stage VI, males and females, also present. This view is substantiated by the fact that the stage VI females showed, not only for total length but also for many of the other measurements, two populations, of which those with the larger mean size were present only to the extent of some 5%. This small population is no doubt a residuum from the larger-sized generation developed earlier in the year (Marshall, 1933). That only a single population of male stage VI was found is not surprising since, as Marshall has pointed out, the evidence indicates that these are shorter lived than the female stage VI.

The material was preserved in 5% neutral formalin as soon as possible after collection, and the measurements and any deductions derived therefrom can strictly speaking only apply to material treated in this way. The measurements were all made with the aid of a projection microscope, the image being projected on to a sheet of paper and the appropriate outlines drawn. The magnification for the metasome segments in the projected figure was × 103, and for the urosome segments and the caudal rami was × 371; the measurements were made from the drawings to the nearest $\frac{1}{50}$ of an inch with a precision steel ruler. One hundred animals of each of the copepodite stages to be considered, namely, stage VI male and female and stage V, were picked out at random from the sample and the following measurements made on the drawings from the projected images (Fig. 1).

From the dorsal side, the length of the head (including fused first thoracic somite) and the length of each of the five free thoracic somites (segments) were measured. The head measurement was taken from the anterior tip to the posterior border, and that of the free segments from the posterior edge of the preceding segment to the posterior border of the segment in question. On the last thoracic segment the length was taken to the centre of the posterior edge of the concave border. The drawings from which these measurements were taken were made with the animal lying flat on its ventral side, and, if necessary, any appendages which hindered keeping the animal horizontal were removed. The body shows a very slight curvature but no correction has been applied for it since tests showed that any correction was extremely small and did not affect the conclusions to be drawn from the results. Strictly speaking, however, the measurements are the lengths projected on to a horizontal plane. Also from the dorsal side the widths at the widest part of the posterior border of all the metasome segments were measured; with the last free thoracic segment this was taken as the distance between the rounded tips. In addition, the head segment was divided into thirds and the width at each third was measured separately.

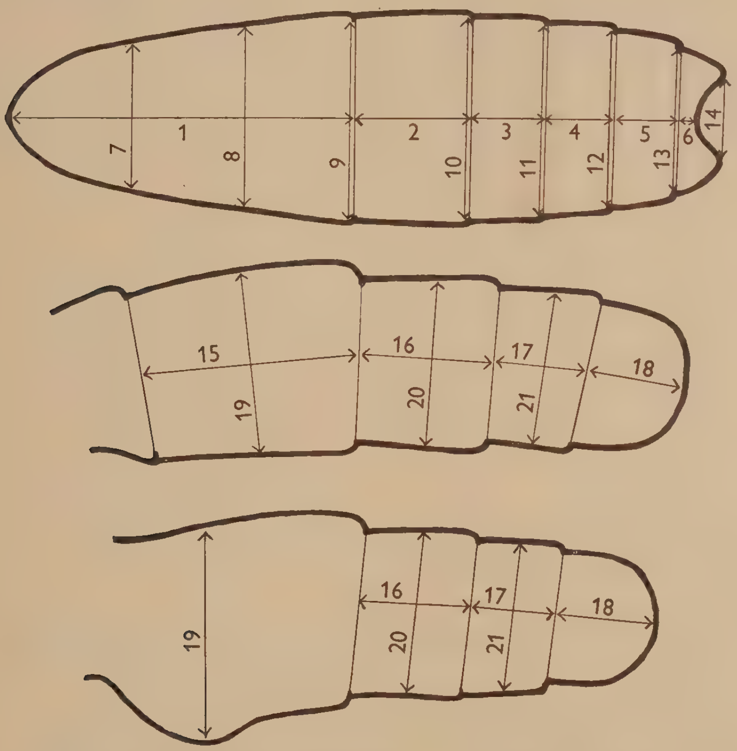

Fig. 1. To show the measurements taken on *Calanus finmarchicus* (Table I).

On preservation, the urosome often undergoes flexion, and all the measurements on this part of the animal were therefore made from drawings made with the animal lying on one side, again removing any appendages necessary in order to place the animal with its dorso-ventral axis horizontal. Even so the length of the first segment of the urosome cannot be accurately drawn in the intact animal, and it has therefore been omitted for all stages. The lengths of the other urosome segments were measured at the centres (posterior border to posterior border, again) so that there are four length measurements for stage VI male but only three for stage VI female and stage V. The depths (dorso-ventrally) at the widest part of each of these segments were also measured, and that of the widest part of the first urosome segment in the female stage VI only (neglected in the length measurements), since here it could be clearly seen. The width of the terminal segment was not measured for any of the stages.

One further measurement was made with the animal on its ventral side, namely, the distance apart of the junction of the caudal rami with the terminal urosome segment.

In order to investigate possible variation in the shape of the head, lines were drawn at five angles to the mid anterior-posterior line from the extreme anterior tip and the length from the tip to where these lines cut the carapace border measured; this was done on the drawings from the dorsal side (see Fig. 2).

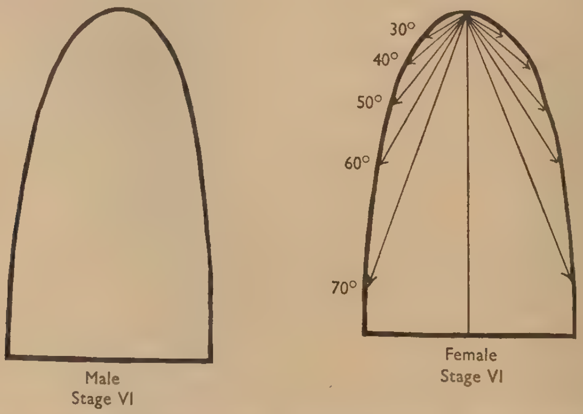

Fig. 2. *Calanus finmarchicus*, showing shape of head in stage VI male and female. Lines set off at angles to mid dorso-ventral line used to determine the shape (see text and Table II).

No evidence was found of any abnormalities such as telescoping of the segments in any of the material under consideration (Gurney, 1931).

In testing the significant differences between mean sizes, the *t*-test was used, the population means and standard errors being estimated from the graphs. For simplicity of discussion the actual units used in the measurements will always be quoted in the discussion of the sizes.

As already stated, a small larger-sized population was shown to be present in the stage VI females; only the smaller-sized and larger population (95 %) is considered in the following.

The Relations of Copepodite Stage VI Male and Female

The male and female stage VI at this time of the season do not differ in the size of the whole metasome obtained by adding together the separate segments (9·70 units males and 9·72 units females, Table I), and this is in accord with the observations of Marshall (1933) who found that the males, although relatively smaller in the early part of the year, were in the summer brood about the same length (metasome) as the females. However, there are differences when individual segments are compared. The most striking difference is that of the head portion: this is much longer in the male (4·91 units) than in the female (4·56 units). With the exception of the first free thoracic segment in which the length of the female does not differ significantly from that of the male, the lengths of the other free segments are all slightly greater in the female, and there is a suggestion of a relative gradient between the two sexes from anterior to posterior, the ratio changing from 0·928 in the head to 1·148 in the last free segment. It will be remembered that for the purpose of comparison within the head itself, the width was measured not only at the posterior edge but also at a distance of one-third and two-thirds its length from the anterior end. There is no significant difference between the male and female in any of these measurements, the head in both narrowing anteriorly.

TABLE I. *CALANUS FINMARCHICUS*. BODY MEASUREMENTS OF STAGES V AND VI

(In arbitrary units.)

	Stage VI		Stage V	
	Male	Female	(i)	(ii)
The metasome				
Length of metasome (by addition)	*9·700*	*9·720*	*8·990*	*8·590*
1. Length of head	4·910	4·560	4·300	3·900
2. Length of first free segment	1·550 (n.s.)	1·568	1·438	
3. Length of second free segment	1·052	1·140	1·047	
4. Length of third free segment	0·924	1·040	0·935	
5. Length of fourth free segment	0·822	0·908	0·810	
6. Length of fifth free segment	0·442	0·504	0·460	
7. Width of head one-third down	2·200 (n.s.)	2·230	2·020	
8. Width of head two-thirds down	2·720 (n.s.)	2·720	2·480	
9. Width of head posterior border	2·860 (n.s.)	2·880	2·670	
10. Width of first free segment	2·818	3·010	2·600	3·000
11. Width of second free segment	2·720	2·840	2·475	2·861
12. Width of third free segment	2·453	2·600	2·195	2·550
13. Width of fourth free segment	2·060	2·155	1·800	2·120
14. Width of fifth free segment	1·183 (n.s.)	1·170	0·920	1·300
The urosome				
15. Length of second segment	2·850	—	—	—
16. Length of antepenultimate segment	1·650 (n.s.)	1·635	2·100	
17. Length of penultimate segment	1·150 (n.s.)	1·160	1·420	
18. Length of ultimate segment	1·295	1·440	1·860	
19. Width of antepenultimate segment	2·600	2·950	—	
20. Width of penultimate segment	2·312	2·360	2·340	
21. Width of ultimate segment	2·215 (n.s.)	2·120	2·105	
22. Width apart of junction of caudal rami	1·000	0·716	0·620	

n.s., differences not significant.

In all the other segments the female is somewhat wider than the male, except in the width between the projecting terminal tips of the last free segment, where there is no significant difference.

The terminal segment of the urosome is significantly longer in the female but there is no difference between the two preceding segments. There is no direct comparison available for the length of the second segment of the male, but it is much larger than the others. As might be expected, the width of the first segment (two fused somites) in the female, which bears the genital opening, is much larger than that of the male, although it should be remembered the comparison is between this and the second somite in the males. The widths of the penultimate and antepenultimate segments show also smaller differences, the former being slightly larger in the male (although not significant at the 0·02 level). The width apart of the caudal rami at their junction with the terminal segment (forming movable joints in the male) is much greater in the males. Indeed this, together with the difference in head length and width of the 'genital' segment of the urosome, are here the most distinguishing size differences in these male and female populations.

THE PRESENCE OF TWO POPULATIONS IN COPEPODITE STAGE V

The first question to be considered is whether there is any differentiation in the stage V animals in the sense that, as regards any of their size measurements, they can be divided into two distinct populations. There is a clear distinction into two populations of about 50% each in total length of the metasome (determined by adding together the separate segments), and this suggests that differentiation into potential males and females, at any rate as regards size, is evident in stage V. This separation into two distinct populations has been confirmed by the analysis of a large number of other catches taken at various times and by the analysis of published data of other workers: the lengths of the stage V metasome can almost always be separated into two populations each composing about half the total. This feature suggests sexual differentiation rather than lack of homogeneity in the material. Such a distinction, however, has not been found in copepodite stages earlier than stage V. It has been unobserved because of the considerable overlap in the sizes. A difference in the size of stage V *Calanus* has often been found in colder regions (Störmer, 1929; Bogorov, 1933), where the modes were quite distinct and has been observed by Ussing (1938) even for stage IV copepodites. In these northern regions, however, the proportions are not constantly 50/50, and the differences are probably due to lack of homogeneity, the two stage V populations having different developmental histories. Thus it has been suggested that the amount of food available at the time of production or even a 2-year development period for one population is concerned. The failure of Marshall (1933) to correlate large and small stage V with males and females in an experimental moult is due to the fact that only small numbers were used and that the differences noted above are population differences.

This differentiation in stage V does not, however, extend to all portions of the animal. It is well marked in the head length (4·30 and 3·90 units), and this might be expected since the difference in the length of the head is the most marked feature in the metasome of stage VI males and females. There is no differentiation in the length of the other metasome segments in stage V, but the widths of all these segments except that of the head are clearly divided into two populations, of which the larger may be presumed to be the female population. There is no distinction in the lengths and breadths of the urosome segments.

RELATION BETWEEN THE SIZES OF STAGE V AND STAGE VI MALES AND FEMALES

There is no feature by which it can be known with certainty which population of stage V gives rise to the males and which to the females. There is considerable growth in the head segment from stage V to stage VI in both male and female; whereas the length of the other metasome segments (of which only one population was present in stage V) shows virtually no increase in the moult to males and only a slight increase in the moult to females. There is no difference in the widths of stage VI male and female heads and therefore it is not surprising to find only one population of these measurements in stage V and therefore the same increases must take place when passing from stage V to stage VI male or female. In the widths of the other metasome segments there are two populations in stage V, one of which is usually larger than the male stage VI and can therefore perhaps be presumed to correspond to the female population; in which case there is little change in the width on passing from stage V female to stage VI female and a moderate change in passing from stage V male to stage VI male. Only a single population was found in the stage V urosome measurements with hardly any change in width from stage V to stage VI. However, the length of all three segments measured in the urosome is much larger in stage V than the equivalent segment in stage VI male or female (as reckoned from the posterior segment). Apparently, therefore, considerable reorganization takes place in the urosome segments at moulting; but it should be remembered that the length of the first urosome segment was not measured.

THE SHAPE OF THE HEAD IN THE TWO STAGES

The mean ratios of the lengths at 40°, 50°, 60° and 70°, set off as described previously, to that at 30°, are shown in Table II for the two stages considered, and in Fig. 2, where the 'average' head is drawn. This ratio, which increases with the angle, is always relatively smaller in the female, indicating that the head is rounder and less pointed than in the male. These lengths were plotted as for the others in stage V, and in each two populations were found present, the ratios derived from which are also shown in Table II. Clearly a round-headed and a more pointed-headed population are present in stage V, and this further strengthens the view that there is differentiation into sexes in stage V. In the 'typical' stage V heads drawn from these figures only the narrow head is found to fit the longest head measurement; and, since the narrow head in stage VI is that of the male, the longest head in stage V may be inferred to belong to the male population.

TABLE II. *CALANUS FINMARCHICUS.* MEASUREMENTS ON THE HEAD OF STAGES V AND VI

	Stage VI		Stage V	
Ratio	Male	Female	(i)	(ii)
Length at 40°/30°	1·65	1·59	1·78	1·54
Length at 50°/30°	2·51	2·36	2·70	2·29
Length at 60°/30°	3·91	3·61	4·21	3·47
Length at 70°/30°	6·75	6·11	7·20	5·80

THE STATE OF THE ANTENNULES ON PRESERVATION

It is well known that the antennules always remain straight on preservation in formalin in stage VI males, but assume some amount of curl, often very characteristic, in the preserved stage VI females. Further, when stage V are preserved, some have curled and some straight antennules. However, it does not appear from the following observations that this can be used to differentiate the sexes in stage V. In the hundred of each stage, picked out at random, all the stage VI males had straight antennules, but so had also sixteen of the stage VI females. In the other females the antennules were curled to a varying degree. In the stage V animals 34% were straight. For all those measurements where analysis had shown that there were two populations in respect to stage V the means were separately calculated for those animals with straight and for those with curled antennules. These means were always the same; there was therefore no separation into two populations as would have been expected had the state of the antennules been correlated with sex, which in turn has been presumed to be the basis of the two population sizes.

Thanks are due to Dr S. M. Marshall for reading the manuscript and for making a number of helpful critical comments on it.

SUMMARY

One hundred each of stage VI male and female and stage V *Calanus finmarchicus* were picked at random, and, by means of a projection microscope, measurements made on a number of segments in both metasome and urosome.

The stage VI female population was mixed containing 95% of a small-sized population and 5% of a larger size-group. The males constituted a single population.

At this time of the season the stage VI male and female metasomes are the same length, but there are differences in the sizes of the individual segments, the most striking of which is that of the head.

The stage V animals in many of the measurements could be quite clearly separated into two size-groups of about 50% each and it is suggested that these are potential male and female populations. This separation at 50/50 has been noticed in numerous other collections of stage V, but it should be emphasized that the differences are population differences in regard to those measurements considered.

Evidence is presented which shows that the state of the antennules after preservation in 5% neutral formalin is unlikely in stage V animals to be a reliable guide to their sex in the next stage.

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J. Mar. biol. Ass. U.K. (1953) 32, 315–318
Printed in Great Britain

PERITROPHIC MEMBRANES IN THE CARIDEA (CRUSTACEA DECAPODA)

By G. R. Forster
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(Text-fig. 1)

Observations on the faecal pellets of various shrimps and prawns were begun after the discovery in 1949 of a thin transparent membrane surrounding the pellets of *Spirontocaris pusiola* (Krøyer). The presence of the membrane in the anterior part of the intestine showed that it could not be secreted by the hind-gut, which is normally very short in the Caridea. Moore (1932) states that the faecal pellets of the *Brachyura* have a thin investment of mucus while those of the Caridea and lower groups have a thin clear outer layer. Subsequently a peritrophic membrane has been described in *Daphnia* (Chatton, 1920) and in the barnacle *Pollicipes* (Batham, 1945). As the membrane found in *Spirontocaris* did not show the properties of mucus, a fuller investigation on its structure and function was made. A membrane has been found in all the Caridea so far examined. *Palaemon serratus*, the common prawn, has been used for many of the tests on account of its size and abundance.

Occurrence and General Observations on the Membrane

A membrane has been observed around the faecal pellets of the following Caridea: *Pandalus montagui* Leach, *Pandalina brevirostris* Rathke, *Hippolyte varians* Leach, *Spirontocaris cranchii* (Leach), *S. pusiola* (Krøyer), *Athanas nitescens* Montagu, *Processa canaliculata* Leach, *Palaemon serratus* (Pennant), *P. elegans* Rathke (= *Leander squilla* (L.)), *Crangon vulgaris* L., and *Philocheras trispinosus* Hailstone.

In *Palaemon*, *Hippolyte*, *Processa* and *Crangon* a membrane has been observed in the intestine as well as around the faecal pellets. The membrane is not always easy to find if the faecal pellets are packed with material, but there are usually small indentations in the faecal strand where it is visible, appearing as a transparent section of the edge of the pellet. When *Hippolyte* is starved for a few days the pellets contain less faecal material and the membrane is clearly visible. Before the animal moults very long pellets are often produced with only slight traces of faecal matter, indicating that the stomach is being completely emptied. To starve *Hippolyte*, and probably any other small caridean, is not at all easy because of its coprophagous habit. The faecal pellets must be frequently removed, as *Hippolyte* and especially *Palaemonetes* can subsist, sometimes indefinitely, without any food by re-ingesting faecal pellets which contain large numbers of Protozoa.

With the larger pellets of *Palaemon* it has been possible to squeeze out the contents and observe the membrane in the form of a thin tube. But usually the slightest touch of a needle will cause the pellet to fragment. The membrane may also sometimes be obtained by itself if the pellets are washed in fresh water: the contents swell, and are slowly extruded from one end of the pellet, or else rupture the membrane. The process can be continued by washing first in dilute and then in more concentrated NaOH solution.

The Nature of the Membrane

Chemical tests have been made on the pellets of *Hippolyte* and *Palaemon* to ascertain the composition of the membrane.

Effect of Acids and Alkalis. The membrane is insoluble in either weak or strong alkalis, and withstands boiling in concentrated KOH solution. This shows that it does not contain mucins or mucoids as these are soluble in dilute alkalis (Lloyd & Shore, 1938).

Concentrated HCl dissolves the membrane rapidly, but a dilute solution has little or no effect.

Chitin Test. As the results given above conform to the properties of chitin (Yonge, 1932), the standard van Wissenlingh test for chitin (Campbell, 1929), critically discussed by Richards (1951), was carried out. This has given positive results for the membranes surrounding the pellets of *Hippolyte varians* and *Palaemon serratus*, both with naturally extruded pellets and with others dissected out from the anterior part of the intestine. A few of the chitin tests have not been positive but this has probably been due to insufficient hydrolysis with KOH. Owing to the small size and delicate nature of the test, pieces must not be allowed to boil, but heating for about 30 min. at 150° C. was found sufficient to obtain a good violet-red coloration with the iodine and sulphuric acid reagent. It was obviously difficult to be absolutely certain that the membrane itself, and not some of its contents, gave the positive reaction. On three occasions, however, pellets were obtained with clear sections of membrane that still gave the colour reaction satisfactorily. Moreover, when one pellet was treated with 3% acetic acid after boiling with KOH, the membrane dissolved as chitosan should, but the contents of the pellet remained. No precipitate of chitosan sulphate was obtained, but this is hardly surprising in view of the minute quantity of material in the test.

The Source of the Membrane

Fig. 1A shows the three regions of the gut. In numerous dissections a membrane has been found only in the intestine (mid-gut), and is assumed to be secreted by the epithelial cells. Sometimes the faecal thread and membrane projected a little way forward from the opening of the intestine, the anterior end being occasionally twisted or rolled up into a coil. This was doubtless brought about by mechanical disturbance during dissection and by anti-peristaltic movements of the gut, as it was scarcely ever observed when the animals were first narcotized with urethane and the intestine separated from the stomach straightaway. Permanent sections were found unsatisfactory for observations on the membrane, which owing to its extreme fragility was nearly always damaged or so tightly pressed against the faecal material as to be indistinguishable from it. Fig. 1 (B, C) shows the junction of the mid-gut and fore-gut. Although at first it was thought that the membrane might be secreted around the dorsal lip, this would scarcely be possible in the presence of large numbers of setae, and no indication of it has been found.

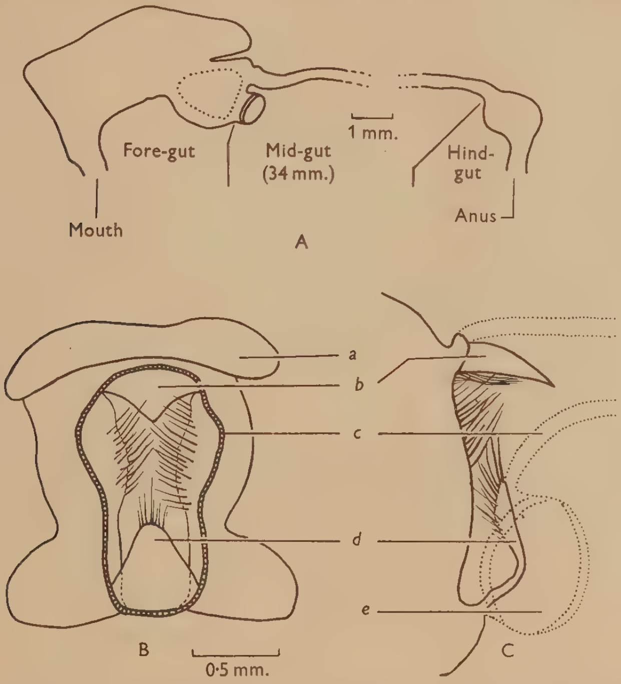

Fig. 1. A, the regions of the alimentary canal of *Palaemon serratus*. The dotted area marks the position of the pyloric filter press. B, the posterior end of the pyloric filter press, looking forwards. C, side view of B. *a*, dorsal caecum; *b*, dorsal lip or valve; *c*, extreme anterior end of the intestinal wall; *d*, chitinous flap (? ventral valve); *e*, opening of the digestive gland duct.

As the membrane is produced in the mid-gut region and contains chitin it may be considered a true peritrophic membrane (Wigglesworth, 1950). The function is probably to protect the epithelium lining the intestine from abrasive material, such as fragments of shells, sponge spicules, and setae which are often found in the stomach-contents of prawns.

Summary

A thin membrane surrounding the faeces of many of the Caridea has proved to be chitinous. The membrane is not secreted by the fore-gut, but almost certainly by the anterior part of the intestine, and is therefore a peritrophic membrane *sensu stricto*.

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J. Mar. biol. Ass. U.K. (1953) 32, 319
Printed in Great Britain

THE SPAWNING BEHAVIOUR OF PLAICE

By G. R. Forster

The Plymouth Laboratory

On the evening of 20 February 1953, two plaice (*Pleuronectes platessa* L.) in the largest tank of the Aquarium were observed spawning. No information on the spawning behaviour of pleuronectids has been found in the literature; nevertheless, the observation may very well not be original, but is considered of sufficient interest to justify an account of it.

The two plaice were swimming in mid-water about 2 ft. 6 in. from the bottom, the female lying slightly diagonally across the back of the male their vents being close together. The female, considerably larger than the male, was quivering violently and emitting a rapid stream of eggs. Mr F. J. Warren who was also watching the tank saw a stream of milt coming from the male. After about 20 sec. the fish separated and settled on the bottom. The eggs were being eaten very rapidly by a shoal of sea-bream (*Pagellus centrodontus* de la Roche). The beginning of the spawning was not seen but the whole act did not take much longer than three-quarters of a minute as the tank had been under observation about half a minute earlier. When captured afterwards the female was found to be almost completely spent, but may have spawned previously. Fertilized eggs from subsequent spawnings by other plaice were taken in the tank for the next 3 days, between 6 and 9 p.m., though the actual spawning was never again observed.

J. Mar. biol. Ass. U.K. (1953) 32, 321–328
Printed in Great Britain

BLOOD AND URINE CONSTITUENTS OF *LOPHIUS PISCATORIUS* L.

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with the collaboration of A. Dujardin

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(Text-fig. 1)

The comparative physiology of glomerular and aglomerular kidneys has provided much valuable information which cannot be ignored in discussion of renal functions. Our knowledge, however, of the exact inorganic and organic metabolism of fishes, particularly aglomerular fishes, is still insufficient. *Lophius piscatorius* L. is the only aglomerular fish whose size permits thorough investigation of such problems. This is the reason why our research on the kidney of *Lophius* starts with an attempt to extend our knowledge in that field.

The habits of angler-fish observed in the Plymouth Aquarium have been described by Wilson (1937).

During a visit to the Plymouth Laboratory in July 1952, when a number of *Lophius* were available, we were able to attempt kidney perfusion. We also took the opportunity of analysing many urine and blood samples from these fish. The samples were taken either on board ship from freshly caught fish, or in the laboratory from fish which had just arrived or spent one night in the aquarium. Others were taken during perfusion experiments.

NITROGEN CONSTITUENTS

Plasma proteins were found, by the Kjeldahl method, to be 39 g./l. in sample L28. A detailed cataphoretic analysis of the plasma of L48 and L49, made in Liège by Dr A. Nizet using Antweiler's microelectrophoretic apparatus, gave the following results: albumin, 6·7%; globulin 1, 14·3%; globulin 2, 46·1%; globulin 3, 32·9% (see Fig. 1).

It thus seems, subject to further confirmation, that the plasma of *Lophius* is not only poor in proteins, but that globulins are largely predominant, which is in keeping with a low osmotic pressure. According to Florkin (1944) the colloid osmotic pressure of teleostean fishes is usually below 20 cm. of water.

Figures in the literature for non-protein nitrogen constituents are very scarce. Denis (1913) gives the following data:

	mg. N in 100 ml.
Total non-protein N	40
Urea	8
Ammonia	3·6
Uric acid	0·9
Creatine and creatinine	5·0

In a pool of heparinized blood samples kept for 24 hr. in cold storage and centrifuged after being used in a perfusion experiment (L 18), we found in the trichloracetic filtrate:

	mg. N in 100 ml.		mg. N in 100 ml.
Total non-protein nitrogen (Kjeldahl)	*21*	Uric acid (Folin-tungstic filtrate)	0·17
Urea (urease in Conway box)	0·85	Creatine	0·21
Ammonia (Hoppe-Seyler, 1930)	0·32	Creatinine	0·28
Trimethylamine + trimethylamine oxide (Hoppe-Seyler, 1930)	11·34		

Sum of analysed constituents *13·17* = 63 % of the total.

In a similar pool (perfusion L 28) we found *total non-protein nitrogen* to be *30* mg. N in 100 ml., of which urea was 1·5.

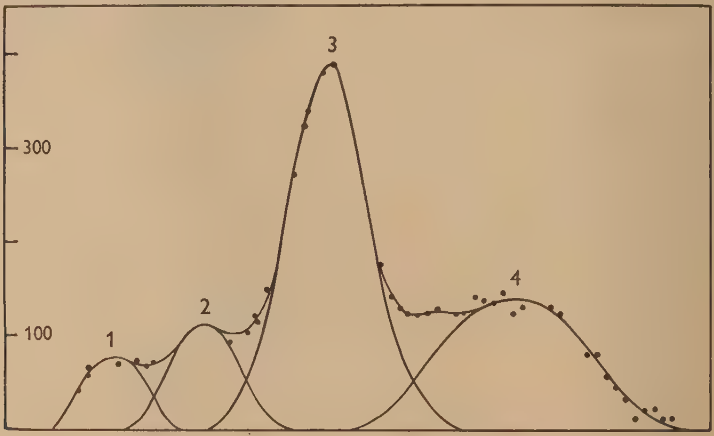

Fig. 1. Microelectrophoresis of *Lophius* plasma (Antweiler apparatus).

In a fresh sample of blood immediately centrifuged and deproteinized with trichloracetic acid (L 48 and 49 mixed), we found 16 mg. of total non-protein nitrogen and only traces of allantoin.

The haemoglobin content of *Lophius* blood is low, the cell volume being only about 17 %. We did not study the oxygen-carrying power of the haemoglobin, nor its absorption curve.

There are no figures in the literature of the amount of allantoin, trimethylamine and trimethylamineoxide in the blood of *Lophius*. In the pool of blood analysed, we found only negligible amounts of urea (much less than was accepted by Denis in 1913). There is very little ammonia, 0·32 (mg. N/100 ml.), as compared with the 3·6 of Denis. We have not yet tried to find whether this ammonia is preformed or not. Creatine and creatinine are not as concentrated as might be expected from the urine figures: 0·26 and 0·28 in plasma, and 51·2 and 4·3 in urine (Table II); therefore, they are highly concentrated by the kidney. Trimethylamine and its oxide comprise 55% of the non-protein nitrogen in the plasma; they are much less concentrated by the kidney than creatin, up to only 28·4 mg. per 100 ml. urine, less than three times the concentration in the blood. Allantoin is practically absent from blood and urine (Table II). We did not have a sufficient volume of well-preserved blood and urine samples for amino-acid determinations; according to Grollman (1929), their concentration would be of the same magnitude in urine and plasma. There still remains in plasma 37% of undetermined non-protein nitrogen, while in urine, where we found 4·5 amino-acid nitrogen, we failed to identify 30% of the non-protein nitrogen. There is still the possibility that an important nitrogen constituent of plasma and urine is as yet unidentified.

TABLE I. NITROGEN CONSTITUENTS OF *LOPHIUS* URINE (MG. N/100 ML.)

		Total non-protein N	Urea	NH_3	Tri-methyl-amine. Total oxide	Uric acid	Creatine	Creatinine	Amino-acids
Denis (1913)	Composite sample of bladder urine, *c.* 1 hr. after death	83	12	1·2	—	0·1	14	0·7	—
Grollman (1929)	Woods Hole	180	1·2	1·0	—	0·8	50·2	7·3	25·8
	Caught in Atlantic	373	0·7	1·3	—	0·4	27·9	4·1	14·3
	Caught in Atlantic	337	0·6	1·0	—	0·2	156·0	8·0	18·0
	From aquarium	56	2·7	0·7	—	tr.	12·9	0·9	4·5
	From aquarium	—	2·7	1·3	—	—	23·0	1·5	8·0
Our determinations	Pool of bladder urines from 20 *Lophius*	148·4	1·6	19·2	28·4	0·03	51·2	4·3	4·5

Notes

According to Grollman, trimethylamine oxide constitutes the greater part of the remaining nitrogen.

According to Denis (1922), the percentage of amino-acids in urine and in blood is of the same magnitude.

Our determinations were from *Lophius* catheterized on arrival at the laboratory or after one night in the aquarium. The methods used were as for plasma, except that amino-acids were determined by a modification of Folin & Wu's method after elimination of NH_3 by permutit.

TABLE II. NITROGEN CONSTITUENTS OF *LOPHIUS* URINE

Bladder urines from individuals, conditions as in Table I.

No.	Total non-protein N	Urea	NH_3	No.	Total non-protein N	Allantoin
L 3	21	2·5	—	L 19	245	—
L 10	82	—	—	L 20	280	—
L 12	54	2·3	2·2	L 21	89	—
L 13	290	—	—	L 22	194	—
L 14	346	5·3	2·1	L 23	140	—
L 16	140	—	—	L 27	56	0·13
L 17	91	—	—	L 36	75	0·15
L 18	89	—	—	L 37	50	—

Total non-protein N in bladder urines of individuals catheterized on deck: LE, 255; LC, 210; LK, 243.

MINERAL CONSTITUENTS

In the following table we have condensed the figures for blood of Marshall (1930) and of Smith (1929), translated into milliequivalents per 100 ml. We reproduce only the limits of variations.

Marshall: Cl, 16·8–22·8.
Smith: Cl, 16·6–20·9; PO_4, 3·2–6·7; SO_4, 1·2–3·7; Na, 18·6–21·8; K, 6·9–9·9; Ca, 2·1–3·9; Mg, 1·0–1·4.

Table III shows the figures obtained in Plymouth in July 1952. Methods used were: Cl, St Russnyak (1926, p. 211); PO_4, Briggs (1922); Na and K, flame photometer; Ca, oxalic precipitation; Mg, colorimeter according to Harold Fister (*Manual of Standard Proced. for Spectr. Chem.*), a few gravimetric controls being made.

TABLE III. MINERALS IN HEPARINIZED PLASMA OF *LOPHIUS* (M.EQUIV./100 ML.) PLYMOUTH, JULY 1952

Nature of sample	Cl	PO_4	Na	Mg	Ca	K	Δ
L 1+2 kept 15 hr. in aquarium under nembut. anaesthesia. Plasma separated after 5 days preservation of blood in cold storage	13·2	—	—	—	—	—	—
L 14 at end of kidney perfusion for 100 min.	14·0	—	—	—	—	—	—
Pool from L 16, 17, 18, separated after kidney perfusion 4 hr. after arrival	16·2	2·3	20·8	—	0·72	—	—
Pool from L 19, 20, 21, 22; 23, 24, 25, 26, 27, 28, 29 (2 days) kept in cold storage and after use for perfusion experiment, lasting 3.30 hr.	16·9	—	—	0·40	—	—	—
Pool from L 33, 34, 35, 36, 37, 38, 39, 40 all well alive 4 hr. after arrival and kidney perfusion	16·1	—	—	—	—	—	—
Pool from L 46, 47, 48 well alive—kept 24 hr. and after kidney perfusion 3 hr.	16·2	—	—	—	—	—	—
Plasma from L 48+L 49 (on arrival)	—	1·8	18	0·50	0·55	0·51	0·84
Plasma from L 30 and 31, arrived in good condition, bled and separated at once	19·6	—	21·7	0·58	—	—	—
Plasma L 32, arrived in good condition, bled and separated at once	19·0	—	21·3	—	—	—	—
Plasmas separated on board off Plymouth:							
L A	15·3	—	18·5	—	—	—	—
L D	15·3	—	—	—	—	—	—
L H	15·6	—	—	—	—	—	—
L E, F, +G	—	1·8	19·3	—	0·67	—	—
L	17·2	—	18·5	—	—	—	—

In one sample of pericardial fluid we found 17·9 m.equiv. chloride.

Our figure for Δ of the plasma, 0·84° C., is the same as the figure given by Smith (1929) at Woods Hole, while Bottazzi (1897) found 0·86 at Naples. In urine we confirm the figures of Smith, who found from 0·66 to 0·85. We found figures varying between 0·60 and 0·88 (Table V). We confirm the view of Marshall (1930) that urine of marine teleosts is either iso- or hypotonic to the plasma, never hypertonic.

Hypotonicity does not mean that certain, inorganic constituents are not highly concentrated by the kidney (Table IV). Mg comes first. Our figures in plasma are about 0·5 m.equiv. per 100 ml. In urine the concentration varies from 22·6 up to 34·5 m.equiv., for *Lophius* catheterized on arrival. On board ship we found similar concentrations, except that two samples contained less, 14·6 and 15·8 respectively.

TABLE IV. MINERALS IN URINE OF *LOPHIUS PISCATORIUS* (M.EQUIV./100 ML.)

Author	Cl	PO_4	SO_4	Na	Mg	Ca	K	Remarks
Denis (1913)	18	0·3	—	—	—	—	—	—
Sulze (1922)	2·1	—	0·7	—	2·5	0·36	—	Naples. Mixtures of urines from several *Lophius*
	2·3	—	1·4	—	3·2	0·42	—	
Grollman (1929)	5·6	0·8	—	—	3·8	0·2	—	Atlantic
	18·8	0·8	—	—	10·8	0·3	—	Aquarium
Smith (1929)	14·1	0·29	—	—	6·3	0·93	0·19	Aquarium (highest and lowest figures)
	20·9	1·47	—	—	10·0	1·52	0·76	—
Marshall (1930)	7·2	—	—	—	—	—	—	Aquarium (highest and lowest figures)
	22·9	—	—	—	—	—	—	
Smith (1929)	14·8	0·6	—	—	5·7	1·18	0·19	Starved in Aquarium
	20·9	0·7	—	—	6·3	1·38	0·27	
	16·1	0·28	—	—	6·4	0·93	0·19	Fed in Aquarium
	20·7	1·47	—	—	10·0	1·52	0·76	

So far as we know, Mg is the constituent of the urine for which the *Lophius* kidney shows its greatest power of concentration. Ca has been shown to be only slightly concentrated, as in man. But as one of us showed (Brull, 1930) there are three different physico-chemical forms of Ca in plasma, one of which is not excreted in urine (bound with proteins), one to a very small extent (ionized Ca), and one very fast and highly concentrated (complex Ca molecules with organic acids); therefore total Ca figures give no idea of renal concentration. The same is true for phosphates; the average plasma figure is below 2 m.equiv. %. In urine, we found from 0·15 to 9·0, which means deconcentration or concentration; but with biological methods and with the isotope ^{32}P, we demonstrated in our laboratory in Liège (Brull, 1927; Govaerts, 1947) that what is considered as inorganic phosphates in the plasma of the dog exists in at least two different physico-chemical forms, one of which only is excreted. It is possible that this phenomenon is interspecific. Potassium figures in plasma are about 0·50 m.equiv./100 ml., and the kidney seems to excrete very little potassium since concentrations in urine are lower than in plasma.

Chlorides in plasma range between 13·2 and 19·6 m.equiv. %; in the literature the variations mentioned are a little wider: 18·6–22·8; in urine, figures published range from 2·1 to 20·9. This might mean that chlorides may be diluted, never concentrated. Our figures for bladder urine range from 0·33 to 19·4, which would have the same significance. But our kidney perfusion experiments have shown that the kidney may slightly concentrate chloride when the plasma concentration is raised. Under normal conditions, this either does not occur or is exceptional.

For sodium in the plasma, figures in the literature range from 18·6 to 21·8. We found the following limits: 18·5 and 21·7; according to Smith (1929) figures for urine are the same, viz. 18·6–20·9; we find lower limits of variations, from 0·4 to 2·3. Sodium is not in line with chlorine. Sulphates in plasma vary from 1·2 to 3·7 according to Smith (1929); Sulze (1922) found from 0·7 to 1·4 in urine; we found higher figures: 7·6, 8·6 and 9·3. This means a concentration of at least three times, although much less than for magnesium.

TABLE V. MINERALS IN *LOPHIUS* BLADDER URINE. (M.EQUIV./100 ML.)

No.	Treatment	Condition	Cl	PO_4	SO_4	Na	Mg	Ca	K	Δ
L 3	Aq. 28 hr.	—	16·2	0·15	—	—	—	—	—	—
L 8	Arr.	—	18·1	—	—	—	—	—	—	—
L 10	Aq. 18 hr.	—	18·0	—	—	—	—	—	—	—
L 13	Arr.	D.	10·8	—	—	—	—	—	—	—
L 14	Arr.	H.	6·2	9·0	—	0·8	—	—	—	—
L 12	Arr.	D.	16·9	0·9	—	—	—	2·0	—	—
L 13	Arr.	D.	10·9	1·0	—	—	—	—	—	—
L 15	Arr. 4 hr.	—	17·0	—	—	—	—	—	—	—
L 16	Arr.	H.	18·4	0·1	—	—	—	1·2	—	—
L 17	Arr.	H.	18·8	0·16	—	—	—	—	—	—
L 18	Arr.	D.	11·6	—	—	—	—	—	—	—
L 19	Arr.	D.	11·7	—	—	—	—	—	—	—
L 20	Arr.	D.	11·1	—	—	—	—	—	—	—
L 21	Arr.	D.	18·7	—	—	—	—	—	—	—
L 22	Arr.	D.	18·1	—	—	—	—	—	—	—
L 23	Aq. 17 hr.	H.	16·9	—	—	—	—	—	—	—
L 24	Aq. 17 hr.	H.	18·8	—	—	—	—	—	—	—
L 25	Aq. 15 hr.	D.	18·0	—	—	—	—	—	—	—
L 27	Arr.	H.	16·4	—	9·3	—	31·0	—	—	0·68
L 33	Arr.	H.	17·0	—	—	—	—	—	—	—
L 38	Arr.	H.	—	—	7·6	—	30·5	—	—	—
L 43	Arr.	H.	13·3	—	—	—	—	—	—	—
L 14	Arr.	H.	4·0	9·0	—	0·8	—	—	—	—
L 32	Arr.	H.	19·4	—	—	—	32·4	—	—	0·74
L 47	Arr.	H.	16·2	—	—	—	—	—	—	—
Pool of bladder urines										
L 3–L 23	—	—	14·7	0·7	8·6	2·3	22·6	1·6	0·2	—
L 31	—	—	—	—	—	—	—	—	—	0·77
L 34	—	—	—	—	—	—	34·0	—	—	—
L 36	—	—	—	—	—	—	31·8	—	—	0·77
L 37	—	—	—	—	—	—	30·6	—	—	—
L 38	—	—	—	—	—	—	30·6	—	—	0·70
L 39	—	—	—	—	—	—	—	—	—	0·84
L 40	—	—	—	—	—	—	30·6	—	—	—
Catheterized on board										
L A	—	—	0·33	—	—	—	—	—	—	—
L D	—	—	7·2	—	—	—	32·5	—	—	—
L E	—	—	7·0	—	—	1·1	14·6	1·0	—	—
L F	—	—	14·8	—	—	1·5	—	—	—	—
L K	—	—	5·1	—	—	—	27·5	—	—	0·60
L I	—	—	7·5	—	—	0·4	19·0	—	0·17	0·88
L G	—	—	3·0	—	—	—	15·8	—	—	—

Aq. 28 hr.: kept in aquarium for 28 hr. Arr.: on arrival at the laboratory. Arr. 4 hr.: 4 hr. after arrival. H., healthy; D., dying.

Several authors suggested that the urine secreted by *Lophius* in the aquarium is not the same as that secreted by fishes living under normal conditions. Grafflin (1931) emphasizes that skin injury in toad-fish increases the concentration of chloride in plasma and urine, while normal urine is poor in chloride and rich in nitrogen. In *Lophius*, taken in 1 hr. trawl, he finds 13·2 and 13·9 m.equiv. %. Preliminary experiments made by one of us (E.N.) tend to show that the rubbed skin of *Lophius* is more permeable, *in vitro*, to crystalloids than normal skin. Grollman (1929) finds lower figures in urine of fish caught at sea, for instance 5·6 m.equiv. against 18·8 in the aquarium, 3·8 mg. sodium against 10·8; for phosphorus the figures are the same; for calcium, 0·2 against 0·3 m.equiv./100 ml. Our figures concern plasma and urine either collected on deck as soon as the fish was brought up, or on arrival at the laboratory after having been kept in running sea water on board.

If we consider the analyses of plasmas separated on board or on arrival, and only take into account plasma separated immediately, we find for chloride the following figures: on board, 15·3, 15·3, 15·6 and 17·2; on arrival 16·1, 19·6 and 19·0. There is therefore a tendency to increase which may explain a similar increase in urine. The same appears to be true for sodium: 18·5, 18·5 and 19·3 on board against 21·7 and 21·3 on arrival.

The Δ for urine of fishes examined on board give us the lowest figure: 0·60° C. and also the highest, 0·88° C., while on arrival they vary from 0·70° up to 0·84° (Table V); for chloride, figures on board vary between 0·33 and 14·8 m.equiv./100 ml., the mean of seven figures being 6·4, while urine from bladders catheterized on arrival in the laboratory gave figures from 4·0 up to 19·6, with a mean of 15·1. For sodium and calcium our figures are insufficient in number. A pool of twenty bladder urines taken on arrival in the laboratory gave 22·6 m.equiv. of magnesium, while the average of fifteen determinations on board was 20·2. Again, on board we have two of the lowest figures: 14·6 and 15·8. More results will have to be obtained before we can really assert that within a few hours after being caught in the trawl there is an increase in the urine inorganic constituents. Our values for chloride, sodium and magnesium tend to confirm the hypothesis of an increased absorption of minerals, at least of NaCl, presumably through the skin.

SUMMARY

The blood of *Lophius piscatorius* is poor in haemoglobin, the volume of red cells being only 17%. The plasma contains less than 40 g. of protein/l., of which only 6·7% is albumin. This explains its low osmotic pressure. As is well known, it contains more crystalloids than mammalian blood, Δ (depression of freezing-point) being 0·84°, the same figure being found at Naples, Woods Hole and Plymouth. This rather high concentration is not due to organic constituents, that of total non-protein nitrogen being of the same magnitude as in mammals; it is mainly due to a high content of sodium

chloride. Chloride is at a concentration of 15·3 m.equiv./100 ml., sodium at 18·5, while in mammals they reach 9 and 15 respectively.

Total non-protein nitrogen concentrations in plasma are similar to concentrations in mammals; the main non-protein nitrogen constituents of plasma are neither urea, ammonia, uric acid or allantoin, but trimethylamine or trimethylamineoxide. Of our analysis 37% of non-protein nitrogen of plasma remain unidentified, so far as we can rely upon our chemical methods. The power of concentration of the kidney for non-protein nitrogen on the whole is not high; it varies up to fifteen times. But the degree of concentration by the kidney, small for most constituents, even for trimethylamine, seems to be very high for creatine which is the main representative of non-protein nitrogen. Of our urine analysis, 30% of non-protein nitrogen remain as yet unidentified.

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J. Mar. biol. Ass. U.K. (1953) 32, 329–336
Printed in Great Britain

BLOOD PERFUSION OF THE KIDNEY OF *LOPHIUS PISCATORIUS* L.

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(Text-fig. 1)

It is well known that the kidney of the adult *Lophius piscatorius* (L.) is practically aglomerular, some of the original glomeruli disappearing late in the young animal, as we have been able to confirm by the examination of fishes a few centimetres long. The kidney of *Lophius* receives only small amounts of arterial blood, its main supply being by venous blood from the caudal vein. Several attempts have been made by previous authors to investigate the secretion of *Lophius* kidney, and to these reference has been made in a paper in which the chemical composition of the blood and urine are dealt with (Brull & Nizet, 1953). We are not aware of publications on blood perfusion experiments on *Lophius* kidneys.

The aim of the present research was to study the secretion of the perfused *Lophius* kidney and its response to variations of venous pressure at the entrance to the organ, and to measure the blood and urine flows with a view to determining whether an aglomerular kidney is sensitive, and to what extent, to variations in its circulation. In a series of previous publications one of us, with his collaborators (Brull & Dor, 1940; Brull & Louis-Bar, 1950, 1953), has investigated the response of the glomerular kidney of the dog to wide variations of blood pressure. The main result of these experiments was that the blood flow through the dog's kidney in the whole animal is to a large extent independent of variations of blood pressure from 100 mm. up to 330 mm. Hg, while the urine flow responds regularly to these variations. Previous research on isolated perfused kidneys of the dog had regularly shown increases of blood flow in response to increases in perfusion pressure.

METHODS

Several favourable conditions had to be realized in order to permit the organization of our experiments: a large supply of good-sized *Lophius*, brought in well alive (so allowing the gathering of sufficient amounts of heparinized blood for perfusion) and with kidneys of sufficient size to permit catheterization of the caudal vein and ureters, etc. These conditions were fulfilled beyond our expectations at the Plymouth Laboratory; and we are much indebted to the Director and Staff of the Laboratory for the generous and efficient way in which they supplied us with *Lophius* in good condition and with any materials which proved necessary for our investigations.

As soon as the fish were brought in, they were killed by a blow on the head, and one of the branchial arteries was cannulated for bleeding into heparin. Several *Lophius* of all sizes up to 100 cm. long were bled, and the blood was kept in cold storage. As soon as more than 500 ml. of blood had been collected, a perfusion experiment was carried out, using a kidney of a large-sized fish. The blood was put in a glass bulb at variable and measured levels above the kidney. Rubber tubing and a glass cannula inserted into the renal end of the caudal vein carried the blood to the gland, while the effluent was collected in a funnel and returned to the bulb. By so doing, the perfusion was made at room temperature, except in one experiment where all the glassware was kept in ice in order that the kidney should be perfused at a temperature of about 5° C.

In Exp. 2 the blood was oxygenated and, after perfusing the kidney, reoxygenated in a large-sized spiral tube down which it flowed in a thin layer, while oxygen ran through in the opposite direction. The degree of oxygenation of the blood was not measured. In Exps. 1, 3 and 4 unreoxygenated venous blood was used.

Urine was collected from the ureter, and microdeterminations were made of total nitrogen (Kjeldahl), chlorine (St Russnyak, 1926, p. 211), and of magnesium (colorimetrically, as described by Fister, 1950).

RESULTS

EXPERIMENT 1

Blood from several *Lophius* was collected the day before and heparinized. The perfused kidney (from L. no. 18, weighing 10·8 kg.) weighed 19 g. Perfusion was started 47 min. after the arrival of the fish. Bladder urine before experiment: 5 ml. with 414 mg. Cl/100 ml. The results are given in Table I. Perfusion pressures are in mm. of blood, blood flows in ml./g. of fresh kidney per minute, and urine flows in ml. per gram of fresh kidney per hour. Room temperature: 24·7° C.

This is the first renal blood perfusion experiment carried out in *Lophius*. We do not know the pressure in the caudal vein at its entrance to the kidney in the live animal, but considering the thinness of the wall, this pressure is likely to be very low. The urine flow began with a blood pressure of 20 mm., and raising this pressure progressively from 40 to 50 mm. up to 120 mm. had no influence on the rate of urine flow. This flow is abundant, and especially so when we consider that about 40% of the kidney is lymphoid and not renal tissue: the rate of flow (per g. fresh tissue) is, indeed of the same order as that in the living dog. The urine is acid (pH 5), and colourless. The kidney does not concentrate chloride, but concentrates total non-protein nitrogen more than five times in sample 3, and three times in sample 5. Magnesium is highly

TABLE I. EXPERIMENT 1

Time (min.)	Perfusion pressure, (mm. blood)	Blood flow (ml./g./min.)	Urine						
			No.	Volume (ml.)	ml./g./hr.)	Cl (mg./100 ml.)	Mg (mg./100 ml.)	N (mg./100 ml.)	
0	20	0·36	—	—	—	—	—	—	Perfusion started (venous blood)
20	—			First drop collected					pH of urine: 5
41	15	0·20	—	—	—	—	—	—	—
42	40	0·48	—	—	—	—	—	—	Pressure raised
60	40	0·52	1	2·0	0·16	594	—	—	pH of urine: 5
73	50	0·65	—	—	—	—	—	—	—
90	50	0·55	2	2·0	0·20	598	—	—	—
93	50→65	0·55; 1·10	—	—	—	—	—	—	—
98	70	—	—	—	—	—	—	—	—
118	70	—	3	2·2	0·25	596	270	113	—
150	70→120	1·10→	4	2·0	0·20	584	255	—	Pressure raised
153	120	4·60	—	—	—	—	—	—	—
180	120	—	5	2·0	0·20	—	222	70	—
188	—	—	6	1·2*	—	—	180	—	Cl in plasma 576, N 23 mg./100 ml.

* Including vol. in catheter.

TABLE II. EXPERIMENT 2

Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g.min.)	Urine					
			No.	ml./g./hr.	Cl (mg./100 ml.)	Mg (mg./100 ml.)	N (mg./100 ml.)	
0	—	—	—	—	—	—	—	Perfusion started (oxygenated blood)
5	55	0·47	—	—	—	—	—	—
10	—	—	—	—	—	—	—	Ureter catheterized: flow starts
20	55	0·26	—	—	—	—	—	Spontaneous drop of blood flow
33	55	0·19	—	—	—	—	—	—
36	55	0·35	—	—	—	—	—	Spontaneous rise of blood flow
46	60	0·32	—	—	—	—	—	—
55	70	0·38	—	—	—	—	—	—
60	80	0·50	—	—	—	—	—	—
70	80→125	1·00	1	0·11	—	—	70	Pressure raised
75	160	1·10	—	—	—	—	—	—
85	190	1·50	—	—	—	—	—	—
108	210	1·40	—	—	—	—	—	—
130	228	—	2	0·13	595	279	—	—
135	228→340	—	—	—	—	—	—	Pressure raised
136	340	2·30	—	—	—	—	—	—
145	—	—	—	—	—	—	—	Blood sample I: Cl in plasma 595 mg., non-protein N 30 mg./100 ml.
160	360	2·40	3	0·16	—	291	—	3 ml. 5 M-NaCl added to the blood
175	—	—	4	0·14	—	—	86	—
180	385	2·50	—	—	—	—	—	—
205	380	—	5	0·14	970	384	—	Blood sample II: Cl in plasma 825 mg., non-protein N: 36 mg./100 ml.

concentrated seeing that *Lophius* plasma contains only about 6 mg./100 ml.: at the beginning of the experiment the urine Mg was about 45 times the plasma Mg, and still 30 times at the end. Thus Mg secretion may be considered as one of the best tests for renal activity in this species. The blood flow, at perfusion pressures between 15 and 40 mm., varies from 0·2 up to 0·5 ml./g. of

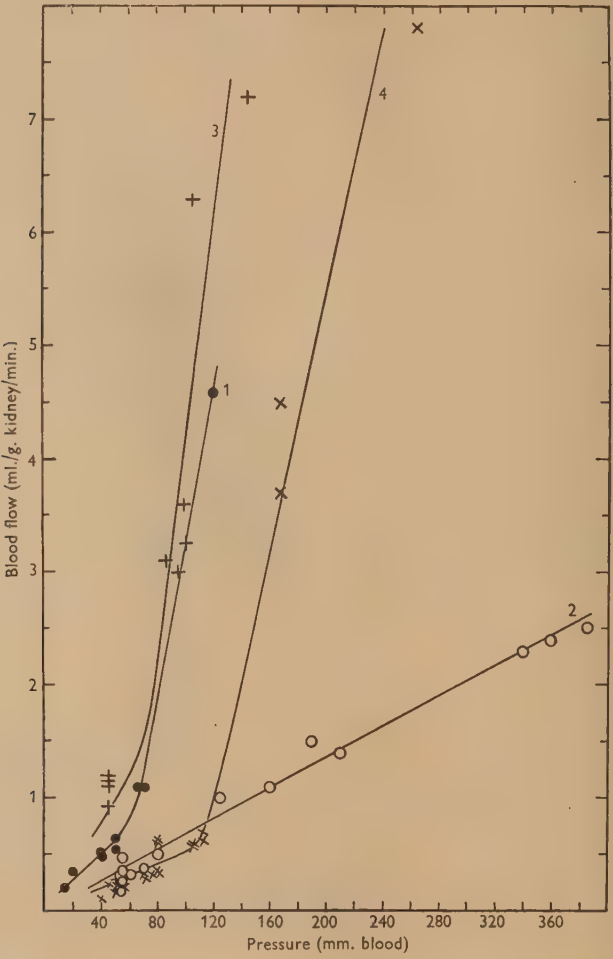

Fig. 1. The relation between blood flow and perfusion pressure in the four experiments described in the text. In Exps. 1, 3 and 4 the kidney was perfused with venous blood, in Exp. 2 with oxygenated blood. In Exp. 4 the perfusate was cooled, its inflow temperature being between 2 and 8° C (Table IV).

fresh kidney per minute, and when the pressure is raised from 40 to 70 mm. the flow increases from 0·5 to 1·1 ml./min. With a higher pressure (120 mm.), the response is proportionally much greater, showing that the resistance of the vascular bed has diminished (Fig. 1).

EXPERIMENT 2

Blood was collected from several *Lophius* just before the perfusion, and the day before. The blood was oxygenated. Room temperature 24·2° C. The perfused kidney (from L. no. 28 weighing 7 kg.) weighed 17 g. The results of this experiment are given in Table II.

The renal blood flow shows spontaneous fluctuations, but follows the pressure more or less passively throughout (Fig. 1). The urine flow increases with perfusion pressure, but only to a very small extent: a 500% increase in pressure is associated with a 25% increase in urine flow. The kidney excretes Cl at the same concentration as it is present in the plasma until the plasma level is raised from 595 to 825 mg./100 ml.; from then on a slight concentration of Cl (970 mg./100 ml.) occurs. Nitrogen is concentrated twice, and this concentration continues throughout the experiment. Magnesium is highly concentrated, as in Exp. 1.

EXPERIMENT 3

270 ml. mixed heparinized venous non-oxygenated blood was used as the perfusate. Room temperature 21·7° C. The perfused kidney (from L. no. 36 weighing 7 kg.) weighed 12 g. Bladder urine before experiment: 20 ml. with Cl. 680 mg. and non-protein nitrogen 75 mg./100 ml. The results of this experiment are given in Table III.

The renal blood flow follows the perfusion pressure, but the variations of flow are proportionally very much greater than the variations of pressure (Fig. 1). In this experiment the urine flow is directly proportional to the perfusion pressure. Chloride in the urine is slightly concentrated from the beginning, and more so after the plasma chloride is raised. Non-protein nitrogen is high in the plasma and is not concentrated. Magnesium is again highly concentrated.

EXPERIMENT 4

550 ml. venous non-oxygenated mixed heparinized blood was used as the perfusate. The blood reservoirs were cooled in ice with sodium chloride. The kidney weighed 23 g. (from L. no. 45 weighing 10 kg. fished the previous day and kept alive in the aquarium). The results of this experiment are given in Table IV.

The blood flow is roughly proportional to the perfusion pressure up to a pressure of about 100 mm.; thereafter the variations of flow are proportionally very much greater than those of pressure (Fig. 1). After the kidney had been subjected to a pressure of 285 mm. of blood, the return of blood flow to its initial value when the pressure was lowered to its initial value (column 1, 290–300 min.) shows that the vessels had not lost their elasticity as a result of this operation. The urine flow, non-existent at the beginning, begins to be appreciable only at a pressure of 70 mm., and at higher pressures there is no parallelism between perfusion pressure and urine flow. Nitrogen is concentrated three times at the most, no more than during perfusion at room temperature. Chloride is not concentrated. Magnesium is highly concentrated, but no more, or even less, than at room temperature.

TABLE III. EXPERIMENT 3

			Urine					
Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g./min.)	No.	ml./g./hr.	Cl (mg./100 ml.)	Mg (mg./100 ml.)	N (mg./100 ml.)	
0	—	—	—	—	—	—	—	Perfusion started (venous blood)
10	50	0·7	—	—	—	—	—	0·2 ml. urine in cannula
15	45	1·15	—	—	—	—	—	Spontaneous rise of blood flow
30	45	0·92	—	—	—	—	—	—
50	45	1·10	—	—	—	—	—	—
62	45	1·20	—	—	—	—	—	—
70	45→85	—	1	0·10	—	—	—	Pressure raised
73	87	3·10	—	—	—	—	—	—
108	101	3·30	—	—	—	—	—	—
115	101→140	—	2	0·20	—	345	64	Pressure raised
120	145	7·20	—	—	—	—	—	—
125	—	—	—	—	—	—	—	Blood sample I: Plasma Cl 670 mg., non-protein N 87 mg./100 ml.
140	145→105	—	3	0·29	760	321	—	2 ml. 1 M-NaCl added to the blood. Pressure lowered
146	105	6·30	—	—	—	—	—	—
152	—	—	—	—	—	—	—	Blood sample II: Plasma Cl 787 mg./100 ml.
165	—	—	4	0·16	—	348	—	—
167	100	3·60	—	—	—	—	—	—
200	100	—	5	0·18	894	378	—	—
210	95	3·00	—	—	—	—	—	—
220	95	—	6	0·17	—	—	—	—
235	—	—	7	0·16	—	—	80	—

DISCUSSION

Lophius kidneys, perfused with heparinized blood, retain for several hours the power of producing urine. This urine is colourless but its chemical composition shows that the gland is actively secreting.

Among the constituents investigated, magnesium was concentrated up to 50 times by the kidney and reached the same degree of concentration as is found in the bladder urine of freshly killed fish. Total non-protein nitrogen is also concentrated by the perfused kidneys, but not so much as in the living fish. Some of the separate constituents are perhaps more concentrated than the figures for the total non-protein nitrogen would suggest: the small volume of the urine samples did not enable us to answer this question. The perfused

TABLE IV. EXPERIMENT 4

Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g./min.)	Blood T (° C) at kidney		Urine						
			In-flow	Out-flow	No.	ml.	ml./g./hr.	Cl (mg./100 ml.)	Mg (mg./100 ml.)	N (mg./100 ml.)	
0	—	—	—	—	—	—	—	—	—	—	Perfusion started (venous blood)
5	40	0·11	5	15	—	—	—	—	—	—	—
25	40	—	—	—	—	—	—	—	—	—	—
35	40→46	—	5	14	—	—	—	—	—	—	Pressure raised
36	46	0·23	5	13	—	—	—	—	—	—	—
50	50	0·20	—	—	—	—	—	—	—	—	—
55	56	0·20	—	12	—	—	—	—	—	—	—
60	70	0·32	2	11	—	—	—	—	—	—	—
65	70	0·32	—	—	1	1·6	0·07	—	110	65	—
70	72	0·28	—	—	—	—	—	—	—	—	—
80	77	0·32	—	10·5	—	—	—	—	—	—	—
85	80	0·35	—	10·5	—	—	—	—	—	—	—
120	81	0·32	—	—	2	2·7	0·12	552	153	75	—
135	105	0·57	—	—	—	—	—	—	—	—	—
165	105	0·58	—	—	3	3·0	0·17	—	170	—	—
180	107	0·62	—	—	4	1·0	0·17	—	176	—	—
185	107	0·69	—	—	—	—	—	—	—	—	—
195	107→162	—	—	—	—	—	—	—	—	—	Pressure raised
200	165	—	2	9	—	—	—	—	—	—	—
205	168	—	—	—	—	—	—	—	—	—	—
215	168	3·70	—	—	—	—	—	—	—	—	—
225	160	—	—	—	5	2·4	0·14	525	170	—	Blood now looks well oxygenated
245	168	4·50	2	9	—	—	—	—	—	—	—
255	166	—	—	—	6	2·2	0·19	—	170	37	—
260	166→230	—	—	—	—	—	—	—	—	—	Pressure raised
270	265	7·80	—	—	—	—	—	—	—	—	—
280	285	—	8	8·5	—	—	—	—	—	—	—
285	—	—	—	—	7	1·8	0·15	—	170	—	—
290	285→45	—	—	—	—	—	—	—	—	—	Pressure lowered
292	45	0·20	—	—	—	—	—	—	—	32	—
300	45	0·16	—	—	8	1·2	0·19	—	196	32	—
	45→80	—	—	—	—	—	—	—	—	—	Pressure raised
305	80	0·60	—	—	—	—	—	—	—	—	—
325	80	0·60	—	—	—	—	—	—	—	—	—
330	—	—	—	—	9	1·2	0·11	508	202	—	Plasma at the end of perfusion: Cl 581 mg., non-protein N 20 mg./100 ml.

kidney excretes chloride usually at the same concentration as in plasma; but when the plasma chloride is artificially raised the concentration of chloride in the urine is higher than that in the plasma.

The activity of the kidney does not seem to be improved by oxygenation of the blood, which is not surprising seeing that venous blood is by far the main supply to such kidneys under normal conditions. Neither is this activity appreciably different at room temperature and at low temperatures close to those at which *Lophius* normally lives. Changes in perfusion pressure up to 100-150 mm. of water raised the urine flow; above 150 mm. there was no effect. This result is in marked contrast with the effects of such changes in a glomerular kidney.

The renal venous vascular net offers but little resistance to the flow of blood: at perfusion pressures between 20 and 40 mm. blood (about 1·5 to 3 mm. Hg) the flow is of the order of 0, 3 ml./g. fresh tissue/min., whereas in the dog the flow at arterial pressures of about 100 mm. Hg is of the order of 3 ml./g. fresh tissue/min. At low perfusion pressures the blood flow in *Lophius* is approximately proportional to the pressure. At higher pressures, however, the flow became proportionately greater in the three experiments in which venous blood was the perfusate, and this change in the slope of the pressure-flow relation occurred at a higher pressure in the experiment (no. 4, see Fig. 1) in which the temperature of the perfusate was lowered. In the experiment (no. 2, see Fig. 1) in which oxygenated blood was the perfusate the blood flow was approximately proportional to the pressure over a very wide range, viz. 50–385 mm. blood; and in both Exps. 2 and 4 the blood flows at a given pressure were lower than in the other two experiments. Further research will show whether the temperature and oxygen tension of the blood play a part in the tone of the kidney vessels of *Lophius* and in their responses to changes in perfusion pressure. In its blood-flow responses to changes in perfusion pressure, therefore, the kidney of *Lophius* behaves, too, in a manner which is in marked contrast with the behaviour of the dog's kidney.

SUMMARY

Lophius kidneys perfused with the heparinized blood (venous) of the fish secrete urine in which total non-protein nitrogen is concentrated, magnesium highly concentrated, and chloride only slightly so or not at all. Oxygenation of the blood, or lowering the temperature of the perfusate from *c.* 20° to *c.* 5° C. does not appear to influence secretion. The blood flow through the kidneys increases with the perfusion pressure, the increase often becoming disproportionately large. The urine flow, on the other hand, above a certain critical level is largely independent of changes in perfusion pressure.

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J. Mar. biol. Ass. U.K. (1953) 32, 337–345
Printed in Great Britain

DECREASED DISCRIMINATION DURING SETTING AFTER PROLONGED PLANKTONIC LIFE IN LARVAE OF *SPIRORBIS BOREALIS* (SERPULIDAE)

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(Text-figs. 1 and 2)

Many larvae of benthic invertebrates search actively for a place to settle, as the end of the planktonic stage approaches. During this process they show considerable powers of discrimination and they can postpone metamorphosis until their search is successful (Thorson, 1950; Wilson, 1952). Since their swimming is feeble and usually undirected, apart from a negative reaction to light in many species, their random explorations must often become very prolonged. Such prolongation will generally be dangerous because planktonic larvae are particularly vulnerable to predators, and because development cannot be long delayed without seriously weakening larvae such as those of barnacles and *Spirorbis*, which do not feed during the searching phase. It therefore seemed possible that searching larvae would become more prone to settle, and consequently less discriminating in their choice of environment, if the planktonic stage were prolonged. Wilson (1952, 1953) has suggested that *Ophelia* larvae behave in this way. Such behaviour would be of adaptive value, in avoiding the dangers of an excessively long planktonic stage. It would also agree with Lorenz's widely accepted idea of instinctive behaviour, as the expression of a conative urge towards a goal, which grows gradually stronger if attainment of the goal is delayed (Thorpe, 1948).

The gregarious reaction of *Spirorbis* larvae (Knight-Jones, 1951) offered a good opportunity for investigating this possibility. These larvae pass successively through a surface-swimming stage, searching stage, setting action pattern and cataclysmic metamorphosis. They were readily obtained by collecting adult *Spirorbis borealis*, epizoic on *Fucus serratus*, during the neap tide periods of larval liberation, and placing them without delay in troughs of sea water. As soon as larvae were liberated in sufficient numbers, they were distributed equally between the various series of experiments in progress, to ensure homogeneity of material. Possible effects, due to diurnal variations in activity, were guarded against by bright illumination at night and by collecting adults and starting experiments at intervals throughout the day and night. Parent *Spirorbis* did not readily liberate larvae at night, but bright illumination and changes of sea water induced some to do so.

LOWERED LEVEL OF SELECTION AFTER PROLONGED SWIMMING

Larvae were removed immediately after liberation, and distributed, about twelve to each bowl, between four batches of glass bowls, each of which had been wiped with cotton-wool just previously, and filled with 250 ml. of sea water. *Spirorbis* larvae rarely attach themselves to freshly wiped glass surfaces, and these were kept for various periods without metamorphosis, until offered the choice of substrata described below.

The four batches of bowls were used in four series of experiments, one series involving freshly liberated larvae and the other three series involving larvae which had been kept swimming for 3, 6 and 12 hr. respectively. In each experiment the larvae were offered a choice between two similar pieces of freshly collected *Fucus serratus*, placed about 6 cm. apart. One piece of each pair, termed the 'previously colonized' piece, had already been exposed to larvae, which had attached themselves to it in numbers varying between 12 and 150, while the other was bare, having been kept under similar conditions, but without larvae. Variation in the numbers of larvae settled on the previously colonized pieces was difficult to control, but in this type of experiment variation within wide limits has been found to have no significant effect on the results, as indeed can be seen from close inspection of the figures in Table I.

A fifth series of similar experiments was carried out using larvae which had been kept from metamorphosis for 24 hr. The majority of such larvae were incapable of locomotion, so those used in this series were obtained by keeping large numbers of larvae in beakers for 24 hr., and then selecting those which still swam.

The numbers of larvae setting subsequently on the previously colonized pieces and on the bare control pieces in the course of each experiment are shown in Table I, together with the angular transformations of the fractions $\frac{\text{no. settled on control}}{\text{total no. settled}} = \sin^2 \theta$ (Fisher & Yates, 1948). This transformation was used to render the variance independent of the mean, and so promote homogeneity in the statistical treatment. Analysis of variance of θ throughout the five series indicates a difference between series, which is significant at the probability level 0·05. Further analysis indicates a highly significant difference ($P = 0{\cdot}01$) between the results from freshly liberated larvae and those from all other larvae. There are no significant differences between the four series of experiments in which these other larvae were used, resulting from the increase in the period of enforced delay from 3 to 24 hr.

In deriving a mean value of θ for each series, it was found as a result of a χ^2 test that the individual larvae could not justifiably be treated as units, perhaps because all those in certain batches had been affected by small inequalities of lighting. Instead it was considered that the most efficient treat-

TABLE I. NUMBERS OF PREVIOUSLY SETTLED *SPIRORBIS* ON PREVIOUSLY COLONIZED/BARE SURFACES, PLUS NUMBERS OF LARVAE SETTING IN EACH EXPERIMENT

Angular transformations $\left(\frac{\text{no. setting on bare surface}}{\text{no. setting on both surfaces}} = \sin^2\theta\right)$ are given in brackets.

	Larvae were kept without surfaces suitable for setting for				
	0 hr.	3 hr.	6 hr.	12 hr.	24 hr.
	69/0+ **8/0** (0)	32/0+ **3/1** (30·0)	32/0+ **1/4** (63·4)	97/0+**6/1** (22·2)	42/0+**0/0** (—)
	28/0+ **6/1** (22·2)	12/0+ **3/0** (0)	83/0+ **2/2** (45·0)	57/0+**3/3** (45·0)	132/0+**0/0** (—)
	25/0+ **7/1** (20·7)	25/0+ **5/2** (32·3)	21/0+ **2/1** (35·3)	53/0+**3/3** (45·0)	15/0+**2/0** (0)
	52/0+ **5/5** (45·0)	57/0+ **5/3** (37·8)	39/0+ **2/0** (0)	25/0+**8/2** (26·6)	83/0+**1/0** (0)
	41/0+**12/2** (22·2)	49/0+ **2/0** (0)	78/0+ **2/0** (0)	51/0+**2/2** (45·0)	45/0+**1/0** (0)
	19/0+**14/6** (33·2)	30/0+**12/1** (16·1)	30/0+**10/2** (24·1)	57/0+**4/5** (48·2)	53/0+**1/1** (45·0)
	30/0+**11/0** (0)	12/0+**14/3** (24·8)	39/0+**10/2** (24·1)	56/0+**1/8** (70·5)	67/0+**7/2** (28·1)
	32/0+**11/2** (23·1)	40/0+**10/8** (41·8)	26/0+ **5/6** (47·6)	77/0+**6/6** (45·0)	83/0+**4/3** (40·9)
	74/0+**12/0** (0)	35/0+ **7/6** (42·8)	61/0+ **7/1** (20·7)	62/0+**2/7** (61·9)	39/0+**0/1** (90·0)
	130/0+ **9/4** (33·7)	87/0+ **5/5** (45·0)	154/0+ **7/5** (40·2)	89/0+**9/2** (25·2)	93/0+**2/1** (35·3)
	51/0+**13/2** (21·4)	75/0+ **6/9** (50·8)	31/0+ **1/5** (65·9)	43/0+**6/4** (39·2)	27/0+**1/1** (45·0)
	91/0+ **7/8** (46·9)	72/0+**10/6** (37·8)	80/0+ **4/5** (48·2)	57/0+**5/3** (37·8)	87/0+**1/1** (45·0)
	81/0+ **7/3** (33·2)	37/0+ **8/6** (40·9)	49/0+**10/9** (43·5)	54/0+**8/2** (26·6)	56/0+**2/0** (0)
	52/0+**13/0** (0)	51/0+**13/3** (25·7)	44/0+ **6/2** (30·0)	65/0+**2/1** (35·3)	38/0+**2/3** (50·8)
	92/0+ **6/7** (47·2)	49/0+ **8/3** (31·5)	43/0+ **5/3** (37·8)	85/0+**1/1** (45·0)	30/0+**2/1** (35·3)
	45/0+**13/1** (15·5)	52/0+ **4/8** (54·7)	54/0+ **8/3** (31·5)	80/0+**3/4** (49·1)	59/0+**3/0** (0)
	33/0+**10/3** (28·7)	44/0+ **2/6** (60·0)	52/0+ **2/3** (50·8)	30/0+**0/0** (—)	44/0+**0/0** (—)
	24/0+**11/3** (27·6)	34/0+ **3/7** (56·8)	44/0+ **2/2** (45·0)	32/0+**1/0** (0)	35/0+**0/0** (—)
	24/0+ **9/3** (30·0)	70/0+ **3/9** (60·0)	39/0+ **0/6** (90·0)	30/0+**1/1** (45·0)	34/0+**0/0** (—)
	40/0+ **8/5** (38·3)	50/0+ **5/5** (45·0)	27/0+ **1/0** (0)	21/0+**0/1** (90·0)	44/0+**1/1** (45·0)
Totals	$\underbrace{192/56}_{248}$	$\underbrace{128/91}_{219}$	$\underbrace{87/61}_{148}$	$\underbrace{71/56}_{127}$	$\underbrace{30/15}_{45}$
Weighted mean θ with S.E. $\pm\Delta\theta$	(25·36 ± 3·35)	(39·31 ± 3·10)	(39·67 ± 3·90)	(41·45 ± 3·43)	(32·33 ± 4·42)
P from $\frac{45-\theta}{\Delta\theta}$	<0·001	>0·1	0·2	>0·2	0·05

ment was to take each experiment as a unit, but to weight the result according to the number of larvae involved, so as to minimize the effect of those batches which had large errors due to small numbers of larvae setting. The weighted mean for each series, with its standard error, is given in Table I. To ensure that this treatment was valid, a more rigorous treatment was also employed, in which each experiment was treated as a unit without weighting: the results were not significantly different from those given in Table I. It is therefore clear that freshly liberated larvae showed well-marked selection of the previously colonized pieces of *Fucus*, whilst larvae which had been kept swimming for 3 hr. or longer showed little evidence of discrimination. Indeed, in some of the series involving prolongation of the planktonic life, the evidence is consistent with random setting. The somewhat anomalous results, obtained from the 24 hr. series, may perhaps have been due to the fact that the larvae of this series had been selected for their vigour, whereas all the other larvae were unselected.

The decrease in numbers setting on the *Fucus*, in experiments which involved delayed metamorphosis (Table I), was due partly to prior setting on the glass of the dishes, but mainly to weakness of the remaining larvae. Their swimming became slower and was occasionally interrupted, until eventually they became entirely immobile. A few showed some of the changes of metamorphosis without becoming attached, but many retained their larval form for several days, remaining motionless and clearly doomed to die unmetamorphosed.

PERCENTAGES OF LARVAE SETTLED, IN THE PRESENCE OF PREVIOUSLY SETTLED INDIVIDUALS AND IN ISOLATION, AFTER VARIOUS PERIODS OF SWIMMING

Choice of substrata which bear previously settled individuals is probably associated with the fact that setting is delayed in isolation (Knight-Jones, 1951). It seemed that the lesser degree of choice exercised after prolongation of the planktonic life might be due to more hasty setting, in gregarious and in isolated larvae alike.

Two parallel series of experiments were set up, using equal numbers of freshly liberated larvae in small beakers, each of which had been wiped just previously and filled with about 50 ml. of sea water. The larvae in one series were distributed so that each beaker contained five, which may be termed 'associated' larvae. Those in the other series were isolated, each beaker containing a single larva. The associated larvae were offered small pieces of *Fucus*, which bore previously settled *Spirorbis*, whilst the larvae of the other series were offered similar pieces of bare *Fucus*. Each of the two series was made up of four similar batches, A, B, C and D. In A the larvae were offered *Fucus* immediately after liberation, but in B, C and D they were kept without *Fucus* until 3, 6 and 12 hr. respectively after liberation. After adding *Fucus*, numbers of larvae metamorphosed were counted at hourly intervals. The experiments were repeated during the liberation peaks of several months, until 800 larvae had been used, comprising a hundred associated and a hundred isolated larvae in each of the four batches.

Fig. 1 shows the curves, representing percentages metamorphosed against time, which resulted from these experiments. In each batch the curve relating to associated larvae is much steeper than that relating to isolated larvae. The difference between the two curves gives some indication of the degree of choice to be expected from each batch in gregariousness tests of the type described above. In batch A, for instance, eighty of the associated larvae settled within 2 hr., whereas only a quarter of that number of isolated larvae

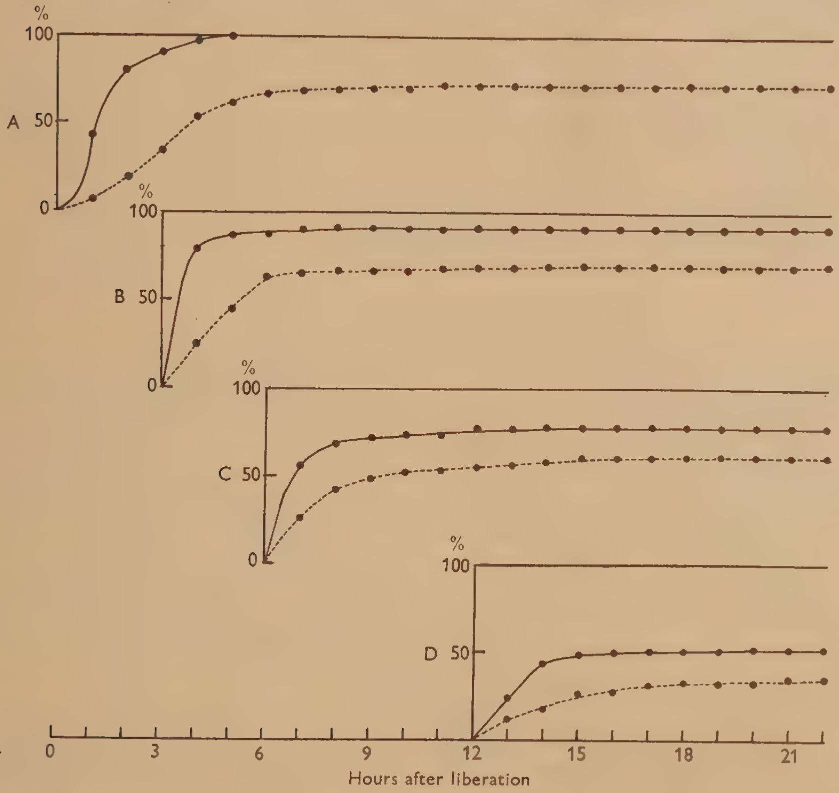

Fig. 1. Percentages metamorphosed, recorded at hourly intervals, in four similar groups of larvae. One group (A) was presented with *Fucus* without delay, whilst the others (B, C and D) were kept without substrata suitable for setting for periods of 3, 6 and 12 hr. respectively. Of the two curves drawn from each group, the continuous line relates to associated larvae, the interrupted line to isolated larvae.

settled within the same time. This agrees very well with the ratio between the numbers of larvae, which settled on previously colonized and bare *Fucus*, in gregariousness tests using freshly liberated larvae (Table I). In the remaining batches the curves are closer together, so the proportion setting in a given time on the bare *Fucus* is considerably higher. In gregariousness tests with similar batches of larvae (Table I) the ratio approached equality, probably because early settlement on the initially bare pieces of *Fucus* made these almost as attractive as the pieces which had been colonised previously.

The fate of the larvae which failed to settle on the *Fucus* (Fig. 1) is shown in Table II (p. 344). Some settled on the glass of the beakers, but when the planktonic stage was prolonged by 6 hr. or more, either because of delay in offering *Fucus* or because of reluctance of larvae to settle on bare *Fucus*, a gradually increasing number of larvae failed to settle altogether, apparently through weakness. Some of these remained capable of swimming for a considerable time, but eventually they all became immobile.

COMPARATIVE RATES OF SETTING

Setting in freshly liberated larvae was initially slow (Fig. 1 A) for larvae were generally photopositive throughout the first 15 min. after liberation and, if conditions did not lead them to settle, for recurrent short periods during the next 2 or 3 hr. Photopositive larvae did not come into contact with *Fucus*. Larvae which had been kept swimming for 3 hr. or longer were generally photonegative and alighted upon *Fucus* very soon after it was presented to them. They then embarked upon the searching phase proper, which involves crawling, interrupted by occasional periods of swimming. Previously they had not crawled, for *Spirorbis* larvae seem unable to crawl upon freshly wiped glass surfaces. Setting in these larvae occurred gradually, not because of difficulty in finding the *Fucus*, but because of individual variation in the length of the searching phase.

Rates of setting were clearly higher in batch B than in A, but seemed lower in C and D than in B. In order to take into account the reduction in the number of larvae available for setting as each experiment proceeded, a plot of the function $\dfrac{\log \dfrac{x}{x-N}}{t}$ was made, where x is the total number of larvae which finally settled on the *Fucus* and N is the number that had settled at time t (Fig. 2). The slope of this curve, $\dfrac{d \log \dfrac{x}{x-N}}{dt}$, gives an individual estimate of the rate of setting, which is independent of the number of larvae available. Considering the associated larvae first, the plot for batch A is a straight line, which intercepts the base-line at about 15 min. after liberation (i.e. at the end of the entirely photopositive phase), and thereafter indicates a constant rate of setting of 0·44 of the population per hour. The plot for batch B is not a straight line, probably because the population is not homogeneous, for setting was delayed in some but not in others. The great majority of these larvae would have settled earlier, if they had had the opportunity, and it seems likely that these settled first, at about double the rate of batch A. The remainder corresponded to those individuals of batch A which were still swimming 3 or 4 hr. after liberation. Their setting was not delayed by the experiment, so it went on at the same rate as in batch A. In batches C and D the rate of

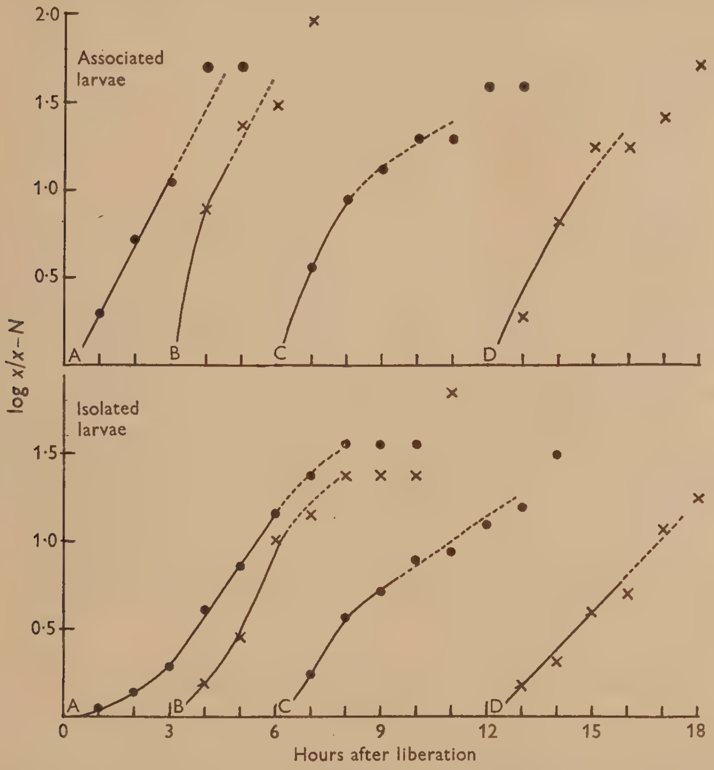

Fig. 2. The results recorded in Fig. 1, treated so as to obtain measurements of the rates of setting which are independent of the numbers of larvae available. A, B, C and D mark the curves relating to each batch of larvae. x = total number of larvae which settled on *Fucus* in each batch. N = number of these which had settled in a given time. The points where N approaches x are inaccurate, being based on small numbers of larvae, so these parts of the curves are dotted.

setting was secondarily lowered, probably because exhaustion, which fatally affected some individuals, was to some extent affecting the majority, but it remained somewhat faster than in A. In each of the curves C and D the initial rate of setting is faster than the ultimate rate, which would be expected if the more vigorous larvae settled first.

TABLE II. FATE OF LARVAE IN THE EXPERIMENTS WHICH FORMED THE BASIS OF FIG. 1

	Associated larvae				Isolated larvae			
	A	B	C	D	A	B	C	D
Settled on:								
Fucus	99	91	78	52	72	70	61	35
Glass	1	9	16	13	15	16	15	18
Failed to settle	0	0	6	35	13	14	24	47
Totals	100	100	100	100	100	100	100	100

The curves relating to the isolated larvae indicate a low rate of setting during the first 3 hr., followed by a somewhat higher rate which was maintained until 7 or 8 hr. after liberation. Thereafter the rate was lower. Probably the food reserves of many larvae become low after 8 hr. of swimming, though there seems to be considerable variation in this respect. Fatal weakness appeared in a small proportion of the larvae which had been kept swimming for 6 hr., yet a few exceptionally vigorous larvae were observed to settle after having been kept swimming for several days. As exhaustion approached the larvae probably found difficulty in completing the minimum searching routine necessary to induce setting, and therefore settled at a lower rate. The movements of many larvae in batches C and D had been observed to be abnormally slow, and they often spiralled aimlessly, or became stuck to obstructions.

Under natural conditions the danger of exhaustion seems slight, for most larvae which succeed in setting probably do so within a few hours. Their negative reaction to light, appearing so soon after liberation, will generally lead them to rocky or algal surfaces before they are carried away from the neighbourhood of the main parent stocks. This and gregariousness account for the fact that *Spirorbis* populations are well concentrated. The brief periods of surface swimming, which recur during the first few hours of searching, give larvae which descend into sandy or muddy coves, or creeks, repeated chances of being carried by currents over neighbouring rocks.

SUMMARY

Laboratory experiments showed that freshly liberated *Spirorbis* larvae settle on surfaces which bear previously settled individuals, in marked preference to bare controls, but that larvae which have been kept swimming for 3 hr., or longer, exercise less choice when setting.

When larvae are freshly liberated there is a great difference between the rate of setting gregariously, and the rate of setting in isolation; but after they have been kept swimming for 3 hr. this difference becomes less, owing to the larvae setting more rapidly both gregariously and in isolation. After swimming for about 8 hr. rates of setting fall off, and many larvae fail to settle, apparently through weakness.

I am greatly indebted to Dr D. J. Crisp for advice regarding this problem, and to him and Mr G. M. Spooner for much help with statistical treatment.

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Printed in Great Britain

A NEW APPARATUS FOR THE COLLECTION OF BOTTOM PLANKTON

By J. Wickstead

From the Plymouth Laboratory

(Text-figs. 1–5)

INTRODUCTION

Many workers have studied the vertical migrations of planktonic animals, and much knowledge has been gained of distribution at different levels at different times of the day. However, with the apparatus used by most of these workers, the plankton in the immediate vicinity of the sea-bottom has had to be neglected.

To sample this bottom plankton, various methods have been devised. Hensen (1895, Plates X and XI) designed a *Wagennetz* which was very complicated, and can have been of little practical use. Russell (1928), Reighard (1894), Beauchamp (1932) and Hardy & Lucas (personal communication) each designed simple and effective nets, but these have the very serious disadvantage of not limiting the samples to the bottom, and there is no way of telling at what level any particular specimen was collected.

The apparatus of Greze (1951) has apparently only been used in inland waters, its effectiveness in the sea being a matter of speculation. Here, precise workmanship is involved, and a finely adjusted mechanism. The latter is to be avoided if possible, since dragging along the sea-bed can so very easily disturb its accurate functioning.

The apparatus used by Bossanyi (1951) effectively restricts the haul to the bottom, but has some disadvantages, i.e. it is heavy and cumbersome, and can be used only in reasonably calm waters and in limited areas where the bottom deposit is suitable.

The apparatus about to be described has been devised to overcome these limitations. It is successful in that it is capable of sampling bottom plankton only; easy to handle, weighing only about 90 lb.; easy to shoot, the shooting not necessarily being restricted to calm weather; simple mechanically; independent of the sea-bed for operating; and self-contained, dispensing with messengers and throttling nooses; and it can be used for making accurately timed hauls.

The principles involved in the working are quite different from those of Bossanyi's net. The idea of using water pressure created over a plane surface caused by towing is not entirely new: Giesbrecht (1893) used it for a mid-water closing net. However, it does not seem to have been used since, certainly not for a bottom plankton net.

I am very much indebted to Mr J. M. Smith, of University College, London; to Captain C. A. Hoodless, D.S.C., and the crew of R.V. *Sabella*; to the Plymouth Corporation, for permission to use their open-air swimming bath; and to Mr P. G. Corbin, whose suggestions and criticisms throughout have been invaluable.

The work was carried out at the Plymouth Laboratory while holding a Colonial Office Studentship.

Description

The apparatus consists of a net, with locks, etc., supported on a rectangular base, 5 ft. 3 in. by 3 ft. 7 in., and mounted on runners. The runners raise the net some 8 in. above the ground (Fig. 1 C).

At the anterior part of the net is a box of thin sheet iron, 24 in. wide, 15 in. high, and 12 in. deep. A 4 in. bucket fits into the cod-end of the net proper. A hinged brass band secures the cod-end to the bucket, and the bucket is attached to the frame by a stout cord.

The net-box is central to the width of the frame, and is flush with the front cross-bar. (Attachment by bolting is used throughout to facilitate such alterations that may have been necessary.) The top of the net-box is supported by two steel strips, bent as seen in Fig. 1 B, leaving a 3-in. gap between the sides of the net-box and uprights of the supports. The back 2 in. of the box is kept free of the supports for fitting of the net proper.

The bearings for the door-hinge are two ½ in. holes in the anterior support, the centre of each hole being 10 in. from the top of the front cross-bar, and 1¼ in. from the leading edge of the support. Straight ½ in. brass tubing serves as the door hinge, held in place by a washer and split-pin at either end. Two slots are cut in the sides of the box to accommodate the hinge.

The door is a sheet of 16-gauge aluminium, 23½ in. by 14½ in., and is bolted directly to the hinge. When in position and closed, it leaves a gap of ¼ in. around the edge, and is about 1½ in. within the box (Fig. 1 C). It is braced behind by stout aluminium angle, a piece from each bottom corner to centre top, and a piece from centre top to centre bottom.

During trials with a small experimental model, it was found important to have the door attached *behind* the hinge.

Outside the net-box two stout levers are attached to the door hinge. From the centre of the diameter of the door hinge to the lever/connecting-arm hinge is 7½ in. About 4 in. of the levers are above the door hinge to act as a counterweight. When the door is vertical, the levers project forward at about 20° from vertical (Fig. 2 A).

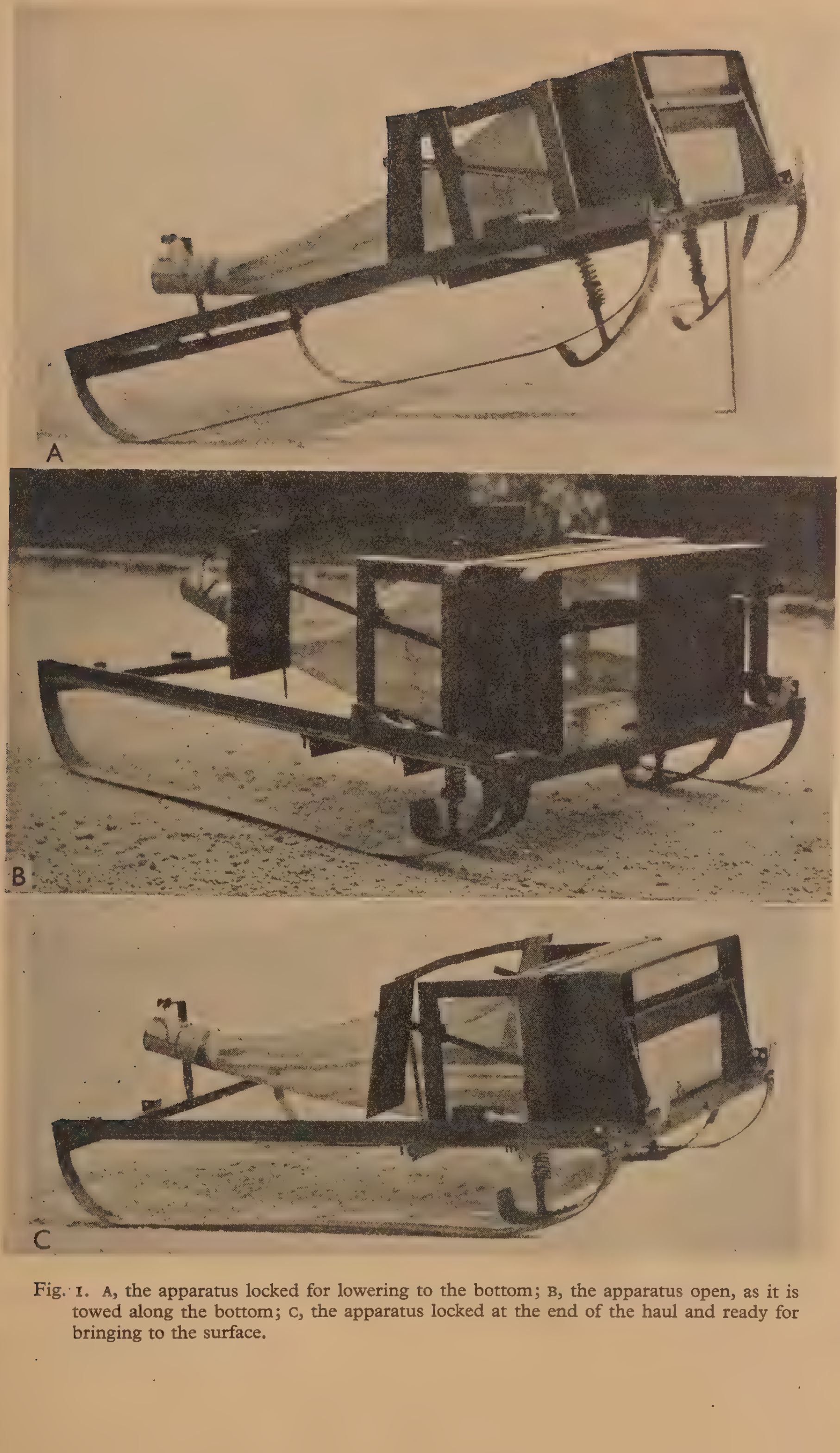

Fig. 1. A, the apparatus locked for lowering to the bottom; B, the apparatus open, as it is towed along the bottom; C, the apparatus locked at the end of the haul and ready for bringing to the surface.

The connecting-arms are ¾ in. angle-iron, each 25 in. long from the lever/connecting-arm hinge to the point of contact with the rollers over which they run (Fig. 2A). The tops of the rollers are level with the door hinge.

The vanes are of 16-gauge aluminium, each 7 in. by 14 in., and are attached perpendicularly to the connecting-arms. All the edges are turned forward for about ¼ in. to increase turbulence, and thereby to increase efficiency. A cross-strut prevents the vanes pivoting laterally on the connecting-arms.

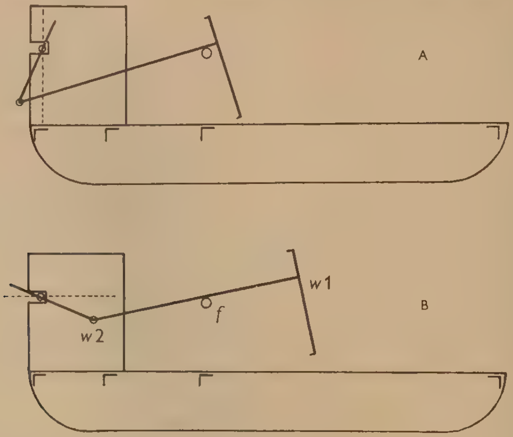

Fig. 2. Diagrammatic side-view of the net: A, closed; B, open. The dotted line represents the door. For explanation see text.

The principle of the apparatus is as follows. When towed through water, the pressure against the vanes and the lower part of the door pushes the vanes backwards. This pulls back the connecting-arms; the connecting-arms pull on the levers, causing the door hinge to rotate through 90°, thus opening the door (Fig. 2B). When stationary, the weight of the door plus levers causes the door to close, pulling the vanes back to the resting position (Fig. 2A). Attachments described below allow the door to be locked when lowered, to open when being hauled, and to be locked when bringing up.

Two small, thin sheet-metal, half-runners, attached to the front cross-bar, project some 2½ in. below the main runners (Fig. 1A). Running up from, and hinged to, the free end of each half runner is a steel bar, bent and doubled back for about 2½ in. at the top (Fig. 3). Guiding the steel bars is an open slot in the second cross-bar, and a closed slot in a small piece of angle steel bolted to the inside of the upright of the posterior net-box support. The steel bars, between the half-runner hinges and the second cross-bar, act as cores to two strong compression springs (Fig. 3). Bolts through the steel bars act as stops against the top of the cross-bar when the springs are at almost their maximum expansion (Fig. 3, *s*). The doubled-over length of steel bar (locking bar).

fits into a slotted piece of iron attached to each connecting arm when the spring expands. The connecting arms are thus locked with the door just sufficiently open to avoid the locking trips (Fig. 1 A).

When the net is being prepared for shooting, the small half-runners are raised, the door fully opened, two strips of metal inserted beside the connecting-arms (see below), the door closed as far as possible, the small half-runners released, the springs pulling the locking bars into the slotted pieces of iron (Fig. 3 A). This action locks the connecting-arms, and therefore the door. The net is shot in this state (Fig. 1 A). On reaching the bottom, the whole weight of the apparatus rests upon the two small half-runners, com-

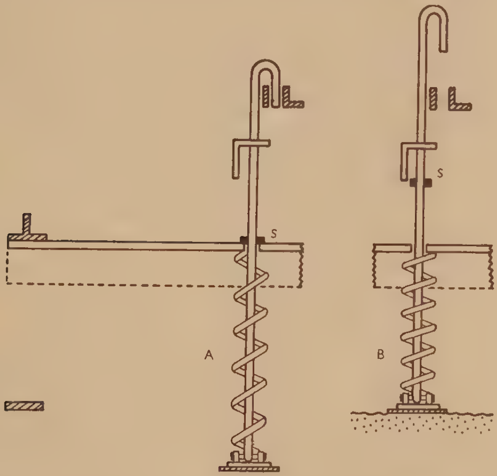

Fig. 3. Operation of the locking bar. For explanation see text.

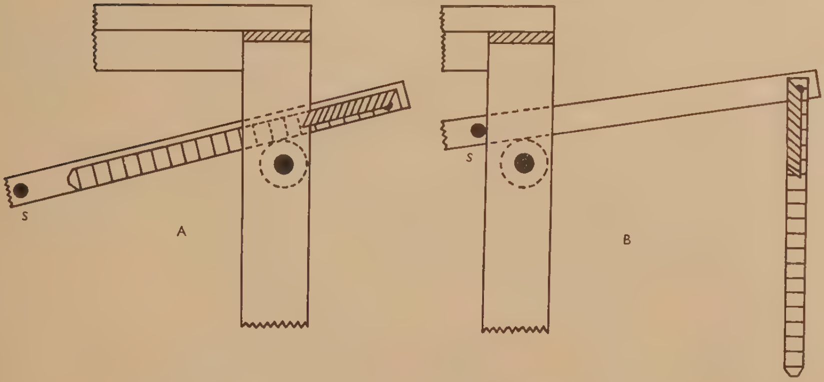

Fig. 4. Function of metal strips. For explanation see text.

pressing the springs until the main runners take the weight. This compression raises the locking bars, thus freeing the connecting-arms, enabling the door to open (Fig. 3B).

Over the bottom of the two small half-runners some fairly stout sheet metal is fixed, to act as 'rubber soles', and prevent the small half-runners from wearing out.

When the connecting-arms are freed, the two strips of metal referred to above prevent the door dropping and locking at this stage. When they are inserted beside the connecting-arms, the turned over length at the end stops the connecting-arms falling right down by catching against the roller supports (Fig. 4A). When the door is almost right open, the two strips, freed of the support of the rollers, fall, leaving the connecting-arms free to close to their fullest extent (Figs. 4B, 1C). The two strips are each pivoted by a small bolt immediately in front of the vanes.

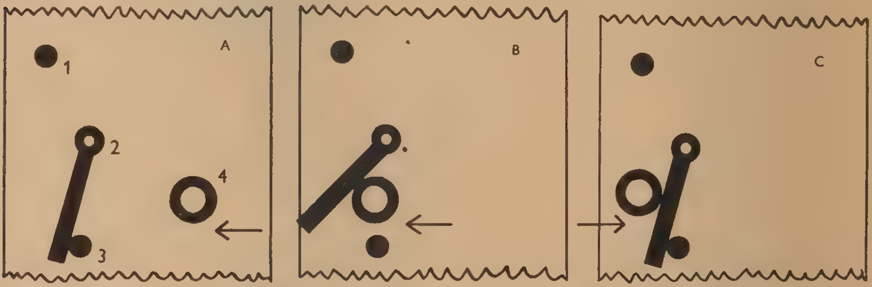

Fig. 5. Detail of trip locking mechanism. A, door nearly shut, the hinge pin is nearing the trip; B, the hinge pin contacts the trip and pushes it up; C, the hinge pin has passed outside the circumference described by the tip of the trip and the trip has fallen back against the locking bolt, preventing the return of the hinge pin. 1, stopping bolt, to prevent the trip reversing; 2, trip; 3, locking bolt; 4, lever/connecting-arm hinge pin. The arrow indicates the direction of movement of the hinge pin.

A stop-bolt in each connecting-arm, acting against the roller supports, prevents the door from going beyond the fully open position (Fig. 4B, *s*).

It will be appreciated that when the door is in the open position, each roller acts as a fulcrum (Fig. 2B, *f*), each connecting-arm a lever with a weight at one end, the vanes (Fig. 2B, *w*1), and a weight at the other end, the door trying to close (Fig. 2B, *w*2). It is very important that these weights should be so disposed that, in the fully open position, the weight of the vanes is only very slightly less than that of the door. If this is done, only the minimum of pressure is necessary to maintain the door fully open and steady.

During experiments in an open-air swimming bath, it was found that the door remained quite steadily open at speeds of less than ½ m.p.h., or about 35 ft./min. This is important since it means that the door will remain fully and steadily open regardless of any irregularities in the towing speed, providing that the speed does not fall below this very low minimum.

When the net has finished its haul, it is stopped, causing the door to fall and engage three locking trips, one at the bottom centre of the door, and one at each lever/connecting-arm hinge (Fig. 1B), in the manner shown in Fig. 5A–C. The projecting lever/connecting-arm hinge pins engage the two outside trips, which are attached to the inside of the uprights of the anterior net-box support.

The importance of having the door bolted behind the door hinge is shown here, since this helps the door to fall beyond the vertical position, so that when the net is being hauled in, water pressure causes the door to be pushed back against the trips and held locked in a vertical position.

SHOOTING

The net can be shot by manhandling it over the side, but it is much more convenient to use a block and tackle. The method now adopted is as follows. The main warp from the winch is attached to the bridles via a ball-bearing swivel. A rope with a hook at the end is passed over a pulley attached to the mizzen boom. The rope is hooked to one of the swivel links. By means of this rope the net is lifted and swung outboard and lowered clear of the ship's side until the main warp takes the strain. This is to make sure that the small half-runners do not touch the side of the ship, thus releasing the connecting-arms. It is then a simple matter to lean over the side and detach the hook. The net is now lowered until it is some 10 or 20 ft. below the surface. For the above operations the ship is stationary. The ship now gets under way at tow-net speed. The net is observed to ensure that it is trailing the right way up, and is lowered. It is lowered quickly at first, but when it gets near the bottom, the warp is let out at about half the rate at which the ship is moving, until the necessary length of warp is out. The ship continues under way until the haul is finished, when a length of some 20 ft. of warp is let out as quickly as possible. This brings the net to a halt and enables the door to close and lock. The ship then heaves to and the net is hauled in. When the swivel is within reach, the rope is hooked on to it, and the last few feet of hauling is done by this. After the bucket has been detached, the net is unlocked and set for the next haul.

There has never been any difficulty in getting the apparatus to land the right way up. This is ensured by the low centre of gravity, the ball-bearing swivel, and having the towing shackles placed about a foot back from the front of the apparatus, near the point of balance, which corresponds with the third cross-bar (Fig. 1C). When being let out, the warp is at about 45° and the net is only about 15° out of horizontal.

The time taken for shooting is about 30 sec.

Discussion

From the outset it was decided that the design must be simple, effective, and self-contained, dispensing with messengers and throttling nooses. As can be seen from the description, all the working attachments are extremely simple, having no intricate mechanisms which may fail to function or function wrongly. Throughout the construction of the apparatus precise workmanship was never needed, and indeed, would have been a waste of time. The cost of construction is therefore very low.

The only part where any particular care is needed is in getting the right balance between the weight of the vanes and the weight of the door when in a fully open position, as described above.

The size of the aperture is a matter of choice for the maker; but the vane area must be increased or decreased accordingly. In the small model already mentioned, the optimum area of the vanes was found experimentally from tests with varying-sized vanes in the open-air swimming bath, where the results could be clearly seen. The vanes were then detached, and a length of cord was attached to the ends of the connecting-arms. This was led over a pulley and attached to various weights until the weight on the cord was sufficient to pull the door fully open. Knowing the area of the vanes, it was possible to calculate what weight of door would be opened by a certain area of vane. Then, by finding the weight necessary to open the door of the big net, the area of the vanes to be used could be worked out. The figure arrived at was $5\frac{1}{2}$ sq.in. for every ounce of weight (approx.). Of course, the pressure of water against the door itself will tend to open it, and this has to be taken into consideration, but the figure arrived at offers a good maximal starting-point.

The opening and shutting of the door rely upon two ever-present factors, i.e. water pressure and gravity. Because of this, the net can be operated on any type of workable bottom.

For quantitative work, hauls can be made of a specified duration since the net can be closed while it is still on the bottom.

There are no obstructions in front of the aperture of the net.

The apparatus can be used in quite rough weather.

All parts, except the lever/connecting-arm hinges, are protected by being within the area of the base frame. The lever/connecting-arm hinges are protected by 'bumpers' (Fig. 1 B).

Different metals are, as far as is possible, prevented from coming into contact by an application of bituminous paint.

In Fig. 1 B & C two attachments can be seen on the back cross-bar. To these can be fitted, when required, a small, coarse-silk net about 9 in. square. The purpose of this is to catch the small members of the benthic fauna which are disturbed by the runners. These are caught in the vortices behind the right-hand vane and so are swept into the net. Small as the net is, it is surprising the numbers of animals that it catches, e.g. Amphipoda, Cumacea, etc.

SUMMARY

A description is given of a new type of apparatus designed to sample plankton from the immediate vicinity of the sea bottom, and purely from this region. Details of the construction are given and the relative merits of the design are briefly discussed.

It could be used for quantitative hauls.

A small additional net serves to sample the smaller benthic fauna at the same time as bottom plankton is being sampled.

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J. Mar. biol. Ass. U.K. (1953) 32, 357–364
Printed in Great Britain

AN EXAMINATION OF THE ORIGINAL SLIDES OF MARINE ACARI OF HODGE, 1863

By H. C. Fountain

(Text-figs. 1–3)

During work on Halacaridae collected in the Plymouth area it became apparent, in view of variations in diagnostic characters among certain species, that it would be useful and interesting to trace and examine as many as possible of the original specimens described and figured by the early British workers in this group of water mites.

Johnston (1836) described *Acarus basteri*, and Gosse (1855) described and figured three species of marine mites, *Halacarus rhodostigma*, *H. ctenopus* and *Pachygnathus notops*. In the same year Hodge (1855) figured a species of the genus *Pachygnathus* (namely *P. seahami*), and remarked that with those of Gosse 'to the best of my knowledge, these are the only recorded British species'. Hodge (1863) described and figured four new species: *Halacarus granulatus*, *H. oculatus*, *Pachygnathus minutus* and *Leptognathus falcatus*. By the courtesy of Mr G. E. Fisher, Curator of the Hancock Museum, Newcastle-upon-Tyne, I have been able to examine Hodge's original slides; these will be described later in this paper. Brady (1875) described four new species, namely *Trombidium fucicolum*, *Pachygnathus sculptus*, *Gamasus marinus* and *Cheyletus robertsoni*. The remaining mites dealt with in his review were *Pachygnathus notops*, *P. seahami*, *Leptognathus falcatus*, *Halarachne halichoeri* Allman, *Halacarus rhodostigma*, *H. granulatus*, *H. oculatus*, *H. ctenopus* and *Pachygnathus minutus*. Of these mites *Trombidium fucicolum* and *Scutovertex minutus* (=*sculptus* Mich.) are oribatids, *Gamasus marinus* is the mite *Halolaelaps marinus* (syn. *glabriusculus*) and *Cheyletus robertsoni* is *C. eruditus*. Of the others he placed *Leptognathus falcatus* in the genus *Raphignathus* Dugés, and Hodge's species *oculatus* and *granulatus* were made synonyms of Gosse's *Halacarus rhodostigma*.

In dealing with Hodge's *Pachygnathus minutus* Brady remarks 'I strongly suspect that it may prove to be an early stage of the following species'. This 'following species' he proceeds to describe as *P. sculptus* n.sp. As will be seen Brady was right in his suspicion and Hodge's *P. minutus* is the larval form of the mite now known as *Simognathus sculptus* (Brady). *Sculptus* therefore becomes a synonym of *minutus*. Brady's conclusion that *Halacarus granulatus* Hodge and *H. oculatus* are the same mite as Gosse's *H. rhodostigma* is not correct in the case of the first, but is true of *oculatus* (see Fig. 1 B, C).

The complete collection of Hodge's slides (eight in number) lent to me by Mr Fisher will now be considered and some of them described and figured. The transparency of the slides varies from perfectly clear and colourless to absolutely opaque. This last state was that of the Type slide of *Leptognathus falcatus*, but after treating it I was able to make a figure of the mite in some detail.

(1) *Pachygnathus minutus*—taken at Seaham. The mountant of the slide is discoloured but transparent. The mite is mounted to show the ventral aspect (Fig. 1 A). The slide bears a label, in addition to Hodge's label, 'See Brady, *Proc. Z. Soc.* 1875, p. 305'. Etched on the slide is the legend 'On Coryne'. The surface (ventral) of the mite presents the appearance of shrinkage into the semblance of a fairly coarse network. The specimen is a larva having only six legs. There are several specimens of the diatom *Grammatophora marina* (Lyngb.) attached to the outside surface of the mite. The dotted lines in the figure indicate the shapes of the pre- and post-dorsal plates which can be seen by 'focusing down'. By the courtesy of Mr E. Browning of the British Museum (Natural History) I was able to compare this slide with one of *Simognathus sculptus* from the Canon Norman collection of Halacaridae in the British Museum, which comparison leaves no doubt as to the identity of *Pachygnathus minutus* with the *Simognathus sculptus* of Brady. The specimens on both Hodge's and Norman's slides were from the Durham coast littoral zone. Of *S. sculptus* Brady, André (1946, p. 138) writes: 'Cette espèce a été rencontrée sur les fonds rocheux de la zone littorale, entre 10 et 65 mètres de profondeur, dans la mer du Nord (Angleterre) et dans l'Atlantique (Le Croisic).'

Of the mite, Hodge himself says, 'Legs smooth, with the exception of the 5th and 6th joints, which have a few short stout hairs. Legs and rostrum irregularly dotted with minute granules; other markings in character. The posterior portion of the body minutely corrugated....This curious species occurred on some stems of *Coryne eximia* from between tide marks, and from the specimen obtained possessing only three pairs of legs it would appear to be a young individual.'

Lohmann (1889, p. 333), in describing '*Aletes*' *minutus* (Hodge) says, 'Was Brady bewog, in dieser Larve ein Jugenstadium seines *Pachygnathus sculptus* zu vermuthen, weiss ich nicht. Anhaltspunkte dafür existieren jedenfalls nicht.' Lohmann evidently had not seen Hodge's slide.

(2) *Halacarus oculatus* (Fig. 1 B)—taken at Seaham. The mountant[1] of this slide has become slightly brown. Like the previous slide it bears an added label, 'See Brady, *Proc. Z. Soc.* 1875, p. 310.'

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[1] Lifting the label mentioned above on the '*oculatus*' slide reveals an inscription probably made by Hodge, scratched by a diamond on the glass, 'Deep Water 26.4.61 Deans Medium'. As already mentioned, the state of discoloration of the mountant varies from clear to opaque. Mr Fisher seemed to consider the mountant to be Canada Balsam, but from this inscription it may be that all are mounted in Dean's medium.

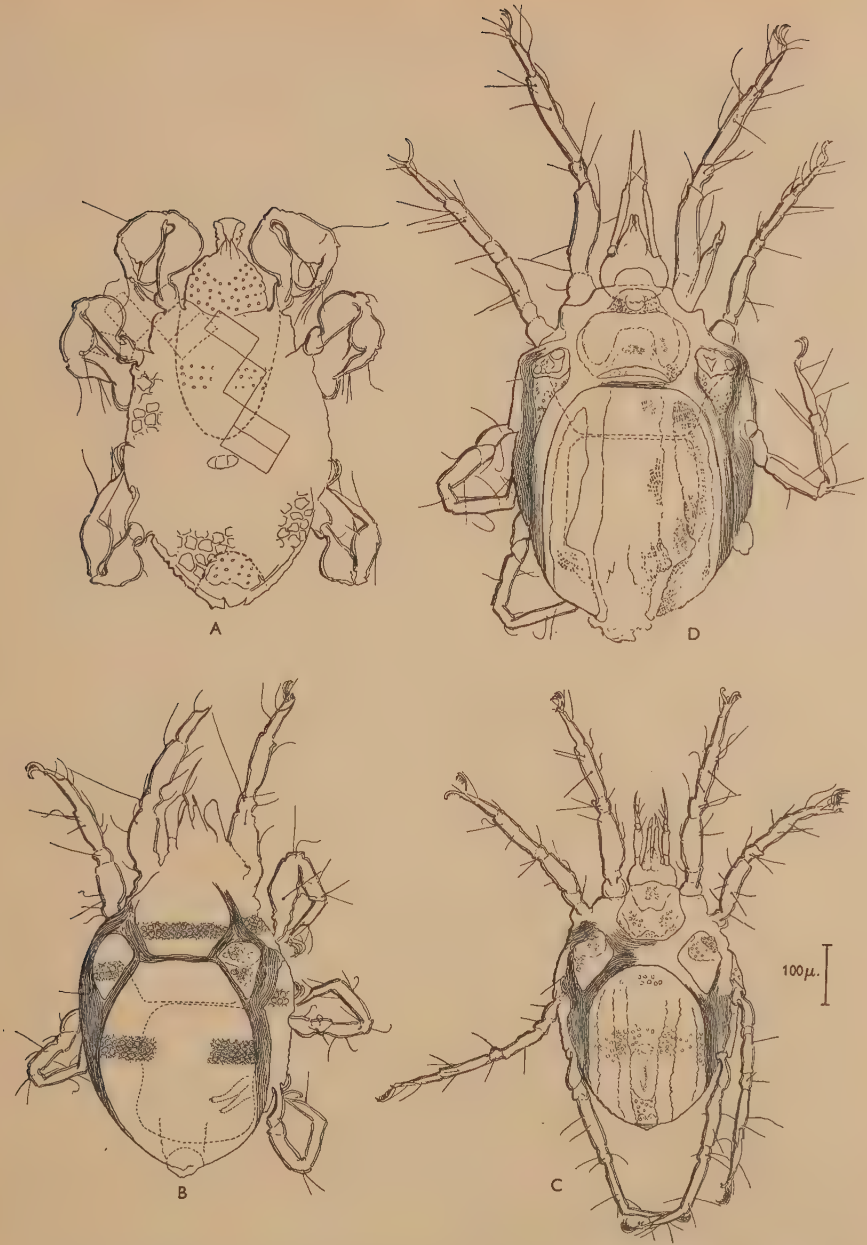

Fig. 1 A. *Pachygnathus minutus*. The larval form of the mite now to be known as *Simognathus minutus* (Hodge, 1863). B, *Halacarus oculatus*. This is the mite known as *Copidognathus rhodostigma* (Gosse, 1855) and must be regarded as an error in identification by Hodge. C, *Halacarus granulatus*. This is the mite known as *Copidognathus glyptoderma*; it must now be called *C. granulatus* (Hodge, 1863). D, *Halacarus rhodostigma*. This is another error in identification and is the mite now known as *Copidognathus gracilipes* (Troues., 1894).

On examination the mite proved to be a definitely different animal from that which is now called *Copidognathus* (*Copidognathopsis*) *oculatus* (Hodge, 1863). Brady, in his review mentioned above, considers this, and the following (*Halacarus granulatus*, Fig. 1 c), to be synonymous with *H. rhodostigma* Gosse; and of Hodge's *oculatus* he says: '*H. oculatus* is, I think, without doubt only the young; and excepting some trivial distinction of surface markings, I cannot find out what Mr Hodge relied on to distinguish his supposed species.'

As the figure shows, the ocular plates do not possess the drawn-out posterior end by means of which the subgenus is at present diagnosed. Unfortunately the slide is not perfect enough to allow a view of the anterior margin of the dorsal plate. Careful examination reveals faint pectination of the claws. As regards this being a diagnostic character it will be well to take account of the following. Newell (1947, p. 150), in writing of *Copidognathus curassaviensis* Viets 1936, says:

> The presence or absence of combs on the claws of *Copidognathus* species is an undesirable primary key character, for although there are many species with clearly pectinate claws, and some which have completely smooth claws (but usually with an accessory tooth), there are many in which the comb is present but difficult to observe, even with oil immersion. In this latter group of species which are apparently in the process of losing the pecten, there are unquestionably some species which show individual variation (e.g. *C. acutus* n.sp.), some specimens having smooth claws, and others having faintly pectinate claws.

André (1946) has also the following footnotes on the same point. On *C. rhodostigma* (p. 90): 'Motas et Soarec ont observé un individu mâle de la mer Noire chez lequel, à toutes les pattes, les griffes sont pectinées et présentent une petite dent accessoire.' On *C. tabellio* (p. 94): 'Motas et Soarec ont observé un échantillon femelle de la mer Noire chez lequel, à toutes les pattes, les griffes sont pectinées et présentent une dent accessoire.' On *C. gracilipes* and *C. gracilipes* var. *quadricostatus* (p. 104): 'Motas et Soarec ont observé deux exemplaires de la mer Noire chez lesquels les griffes présentent non seulement une dent accessoire, mais aussi un peigne.'

I have had the privilege of comparing this specimen with a slide of *C. oculatus* kindly loaned me by Dr Karl Viets of Bremen and also with slides of the same sent to me by Dr Hobart of the Department of Agriculture and Forest Zoology, Bangor. In Hodge's slide it will be seen from the figure that the anterior border of the body is not distinct and the chief features used in deciding that this specimen is *rhodostigma* are the absence of the posterior elongation of the ocular plates, the strongly developed hair of the 3rd segment of the first legs, the very narrow space between the two dorsal plates and the even sculpturing of all the plates. The sculpturing of the ventral plates could not be made out. The size of the mite as mounted is length approx. 360 μ, breadth 230 μ. In a letter on the subject of these 'old' slides Dr Viets says: '...only the Hodge slides may be type specimens of his species, I think'. It seems that we must regard this specimen as wrongly identified by Hodge, and Lohmann's (1889) description, which is clearly that of the *oculatus* of to-day, must be taken as the description of the type.

(3) *Halacarus granulatus* (Fig. 1 C) taken at Seaham. This slide, as the two previous, bears a label, 'See Brady, *Proc. Z. Soc.* 1875, p. 310'. This specimen is undoubtedly the species now known as *Copidognathus glyptoderma* Trouessart. Trouessart's species must therefore sink and the type of the genus *Copidognathus* must be renamed *C. granulatus* (Hodge). The size of the mite is 480 × 358 μ. The sculpturing of the posterior dorsal plate, with its four longitudinal bands, is as shown in the figure of *C. glyptoderma* in André, while that of the anterior dorsal plate is a good approximation allowing for some slight deterioration of the mountant. The suggestion of three sculptured areas which André calls 'trois impressions saillantes disposées en triangle', and the subquadrangular shape of the anterior dorsal plate are also in agreement. Of the ocular plates André's description is 'subquadrangulaires, assez grandes, arrondies à leur angle antérieur et se prolongeant en arrière par une pointe bien marquée. Elles portent chacune une grande cornée antérieure et deux beaucoup plus petites, rudimentaires, en arrière de la précédente.' These two rudimentary cornea are not visible on Hodge's specimen, and although André mentions them they do not seem to be shown in his figure. The posterior dorsal plate is separated from the anterior dorsal plate by a band of dermal tissue about 2½ times as broad as long (André says, 'deux fois plus large que haute'). The general oval shape, the very strong pectination of the claws and the minute denticles on the mandibles also agree with André's description, leaving no doubt that this specimen is the *C. glyptoderma* of Trouessart.

(4) *Halacarus rhodostigma*, 20 fathoms, 16 May 1861 (Fig. 1 D). This specimen is, according to present-day diagnosis, wrongly identified, and is a specimen of the species known as *Copidognathus* (*Copidognathopsis*) *gracilipes* (Trouessart, 1889). Dr I. M. Newell (1947) has placed this species in a new subgenus, and on p. 34 writes:

> ...there is a group of species which formerly were placed in the subgenus *Copidognathopsis*, but which are distinct from all other species of *Copidognathus* in the number of ventral setae on tibia I. The other species of *Copidognathus* are remarkable for the apparently absolute constancy of the number (three) and arrangement (two ventromedial; one ventral or ventro-lateral) of the ventral setae on the first and second tibiae. But in *C. arenarius* n.sp., *C. submarinus* n.sp., *C. gracilipes gracilipes* (Trouessart, 1889), *C. gracilipes quadricostatus* (Trouessart, 1894) and *C. gracilipes largiforatus* (Trouessart, 1889) there are two pairs of ventral setae on 1–5 in both the deutonymph and the adult. These five forms are more closely related to each other than to any species in the genus and therefore form a distinct and natural species group. All have a short, blunt, rostrum which extends only a little beyond the middle of P-2, and apparently all lack rosette pores on any of the plates. The above five forms are removed to

> *Arhodeoporus* n.subg. The name indicates the absence of rosette pores, but this is not the most important character on which the subgenus is based. Furthermore, not all 'arhodeoporose' species belong here.

Concerning this new subgenus of Newell's, Viets (1950) makes the following remarks:

> Newell 1947 trennt nun subgenerisch von *Copidognathus* (*Copidognathus*), nach ihm die Arten mit 3 (unpaaren) Ventralborsten an den I.B.5 umfassend, als *Copidognathus* (*Arhodeoporus*) die Arten ab, die 4, also paarige Ventralborsten an den I.B.5 tragen. Diese Unterschiede und andere unterschiedliche Merkmale (in der Plattenstruktur, p. 35 'apparently (sic!) all lack rosette pores' und in der basalen Breite und Länge des Rostrum relativ zur Palpenlänge) sind subgenerisch mindestens ebenso unbefriedigend, wie die immerhin messbaren Merkmale und Unterschiede in den Okularia. Da die ganz charakterischen Okularia der neben *Copidognathus* (*Copidognathopsis*) *gracilipes* (Trt. 1889) von Newell in *Arhodeoporus* gestellten Arten *arenarius* New. und *submarinus* New. typische Merkmale von *Copidognathopsis* zum Ausdruck bringen, vermag ich in *Arhodeoporus* New. nur ein Synonym zu *Copidognathopsis* zu sehen.

In the Hodge specimen the two pairs of ventral setae can be seen on the right 1–5, the short blunt rostrum is evident and a careful examination, using a 4 mm. apochromatic objective, fails to reveal any rosette pores. In all respects the mite also agrees well with André's description of *C. gracilipes*; the ocular plates and the dorsal plates with their distinctive sculpturing being especially convincing. The size of the specimen is 450 × 280 μ.

(5) *Halacarus rhodostigma* (Fig. 2). The mountant of this slide is dark brown in colour, but as the figure shows the mite is fairly visible and but for the faint pectination of the claws it agrees as far as it is possible to judge with the present *Copidognathus rhodostigma*. The view is ventral and the shapes of the dorsal plates are indicated by dotted lines. The specimen is a male, size 412 × 211 μ and the sculpturing and general appearance agrees with André's description.

(6) This slide (not figured) is Hodge's slide of *Pachygnathus seahami* and is labelled 'Type'. Added inscription on the label is 'Rocks 1861'. There are six specimens on the slide and, although the mountant is perfectly clear and unstained, the specimens themselves are opaque for the most part. The terminal segments of most of the legs in each specimen are visible and are really the only characters it is possible to use for identification. Using these and the faint indication of the provisional genital area in some specimens I make them out to be:

	Length (μ)	
(1) Adult	425	All four medial claws large
(2) Adult	430	All four medial claws large
(3) Adult	475	All four medial claws large
(4) Deutonymph	340	Medial claws 3 and 4 small
(5) Deutonymph	370	Medial claws 3 and 4 small
(6) Deutonymph	335	Medial claws 3 and 4 small

All are the mite now known as *Rhombognathus* (*Rhombognathopsis*) *seahami*.

(7) This slide is also labelled *Pachygnathus seahami* and is of a very damaged specimen. There is only one specimen on the slide and the condition of the mountant is perfect as in the last slide. On the end of the glass slip is etched 'Deep Water 1860'. Using the same features as in the last slide I have no doubt of its being a true *seahami*.

(8) *Leptognathus falcatus* Hodge 1860. This mite (Fig. 3) is labelled 'Type'. When sending me this slide Mr Fisher said in his accompanying letter, '...and *Leptognathus falcatus*, Seaham. Unfortunately the balsam of the latter slide has disintegrated so that the specimen will be of very little use.' A prolonged soaking in xylene had no effect on the mountant and a similar treatment with chloroform met with no more success. This seems to indicate that the mountant was *not* Canada Balsam but almost certainly Dean's Medium. The slide was then immersed in cellosolve and 'forgotten' for about 18 months. It was then possible to see the amount of detail shown in Fig. 3. Because of the danger of destroying the specimen by moving the cover-glass the drawing was made in instalments; the slide being returned to the cellosolve when

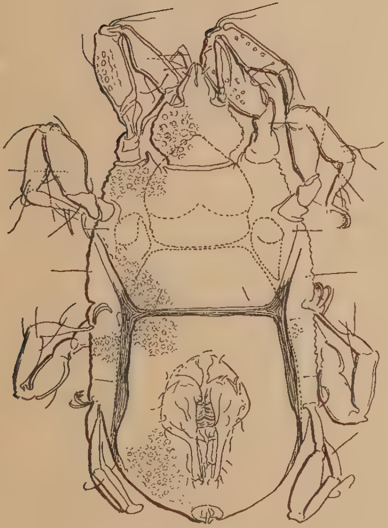

Fig. 2

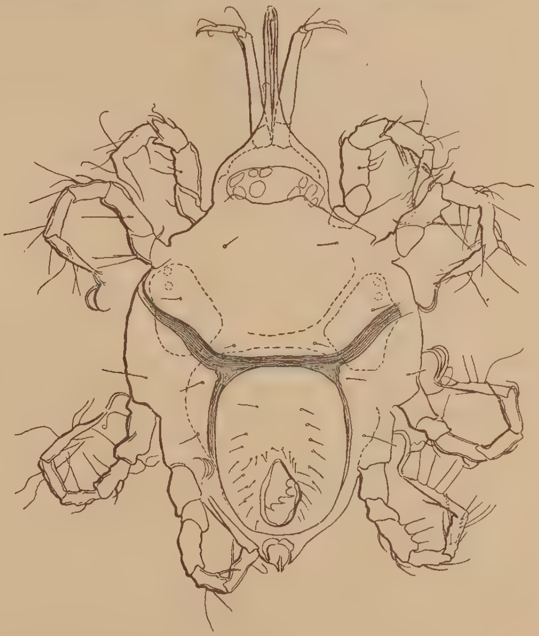

Fig. 3

Fig. 2. *Halacarus rhodostigma*. This mite was correctly identified by Hodge and the figure is given for comparison with Fig. 1 D.

Fig. 3. *Leptognathus falcatus*. Hodge's *type* slide. This mite is now *Lohmanella falcata* (Hodge, 1863).

drying-up of the liquid was reaching the danger point. This is the condition of things at the time of writing. The legend 'Deep Water March 1861' is scratched on the slide by means of a diamond point. The details shown in the figure were obtained by the use of 'phase-contrast' methods and I think the figure is sufficiently detailed to confirm the identity of the specimen with the animal now known as *Lohmannella falcata* (Hodge). The aspect shown is as mounted (ventral) and as much of the dorsal plates and the ocular plates as could be made out is indicated by dotted lines in the figure. The specimen is a female, size 605 × 345 μ, and the genital opening is 66 × 40 μ.

For help and advice in writing these notes, as well as for material for comparison, I have to tender my grateful thanks to Dr K. Viets of Bremen, Dr J. Hobart of the Department of Agriculture, Bangor, and Mr E. Browning of the British Museum (Natural History, Arachnida). Especially have I to thank my friend Dr F. A. Turk for much guidance and for reading this paper, and also Mr Fisher of the Hancock Museum, Newcastle-upon-Tyne, for his patience and kindly replies to all my queries.

SUMMARY

An examination of Hodge's original slides of marine Acari shows that three alterations in synonymy are called for: (i) *Simognathus sculptus* (Brady, 1875) to become a synonym of *S. minutus* (Hodge, 1863); (ii) *Copidognathus oculatus* Hodge, 1860 to become *C. oculatus* Lohmann, 1889; (iii) *C. glyptoderma* Trouessart to become a synonym of *C. granulatus* (Hodge, 1863). Observations were also made on the permanence of Dean's Medium as a mountant for small arachnids and some quotations and observations on the value of certain diagnostic characteristics of the genus *Copidognathus* are made.

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J. Mar. biol. Ass. U.K. (1953) 32, 365–366
Printed in Great Britain

CHLOROCRUOROPORPHYRIN: A SIMPLE METHOD OF PREPARATION

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Munro Fox (1926, *Addendum*, p. 219) described a method of preparation of chlorocruoroporphyrin from the blood of the marine polychaete worm *Spirographis*. This was based upon the very troublesome method of Nencki & Zaleski (1900), which is time-consuming and wasteful. Furthermore, *Spirographis* is not easily obtainable in this country. Lemberg & Parker (1952) started with protoporphyrin, and by careful oxidation with potassium permanganate in acetone obtained a solution which gave mixed crystals of chlorocruoroporphyrin and diformyldeuteroporphyrin esters. Purification was by fractional chromatography. The procedure to be described here is simple and rapid, and could easily be carried out in one day by a biologist requiring a small sample of the porphyrin. The two methods mentioned above would not come into this category.

This work was done at the Plymouth Laboratory, and I am very grateful to Mr T. R. Tozer of that laboratory for so kindly obtaining the animals for me and extracting the blood.

The polychaete worms *Myxicola infundibulum*, *Sabella pavonina* and *Branchiomma vesiculosum* are all readily obtained from the muddy shores of the Salcombe estuary, especially on the Salstone. Blood was taken directly from the vessels of about 100 assorted worms by syringe, and squirted as it was removed from the worms into a mixture of peroxide-free ether (3 parts) and 'Analar' glacial acetic acid (1 part). After all the blood had been added, the mixture was well shaken and allowed to stand in the ice-chest for 30 min. The acetic acid was then washed out with distilled water, the first few washings containing a little sodium acetate to avoid washing out of the pigment, and the remaining solution of chlorocruorohaematin dried roughly by filtering through ether-soaked paper into a distilling flask and evaporated to dryness. 100 ml. of a 50% solution of hydrazine hydrate in glacial acetic acid were then added, and the mixture heated on a water-bath at 90° C. for 10 min. During that time, the chlorocruorohaematin dissolved, and the solution became violet-red and red-fluorescent to ultra-violet light, indicating the formation of the free porphyrin. When cool, the solution was poured into a large separating funnel with 300 ml. peroxide-free ether, and the flask rinsed out with ether, the rinsings being added to the funnel. The acid and hydrazine hydrate were then washed out with distilled water, the first washings containing sodium acetate as before, and the ether solution of the porphyrin shaken with 10 ml. lots of 5% (w/v) HCl (137 ml. conc. HCl/l.) until no more of the lower layers were fluorescent. The pooled acid extracts were then added to ether in a separating funnel, and the porphyrin driven back into ether by the addition of saturated sodium acetate solution—just *red* to Congo Red paper in the lower layer. The ether solution was washed with distilled water to remove salts and acetic acid, and dried by the usual method of filtering through ether-soaked paper. The ether was then distilled off on the water-bath to a volume of about 25 ml., and the remainder evaporated in a dish to dryness. The residue was dissolved in the minimum amount of dry chloroform and when hot, the porphyrin precipitated by the addition of light petroleum, B.P. 40–60° C. This is quicker than the crystallization method, but does not give so fine a product. The precipitate was centrifuged down and washed with petroleum ether, and dried in the incubator. Yield, 22 mg.

Absorption spectra were measured with the Hartridge Reversion Spectroscope:

Chlorocruoroporphyrin	I	II	III	IV
In pyridine (mμ)	645	571	553	517
		I	II	
In 5% (w/v) HCl (mμ)		615	556	

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J. Mar. biol. Ass. U.K. (1953) 32, 367–384
Printed in Great Britain

EXPERIMENTS WITH RADIOACTIVE STRONTIUM (^{90}Sr) ON CERTAIN MOLLUSCS AND POLYCHAETES

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(Text-figs. 1–5)

INTRODUCTION

The use of radioactive strontium in the study of invertebrate metabolism is as yet not extensive; it has been employed successfully by several workers on the metabolism of bone. In both vertebrate and invertebrates calcium is the more important element and strontium, if present, is only in traces, except for the aberrant group of radiolarians, the Acantharia, in which the skeleton consists almost entirely of a salt of this element. Radiocalcium would be the ideal choice for these studies of metabolism, but the radioactive isotope of calcium, ^{45}Ca, has been available up till now only in small quantities and with weak activity. Chemically, and to some extent physiologically, however, strontium replaces calcium (McCance & Widdowson, 1939). Pecher (1941*a*, *b*) has shown that in mice, rats and man the metabolism of the two elements is similar qualitatively, though there are quantitative differences: both are concentrated in the skeleton, with very little in the soft tissues, but the uptake of calcium is greater than that of strontium. Resemblances in metabolism of the two elements in vertebrates are also demonstrated by Posin (1942) and by Erf & Pecher (1940). Thus it is because of their similar behaviour, and the availability of radioactive strontium which is carrier-free, that this element has been of interest as a tracer for the study of calcium metabolism in mammals. It is also employed by Swann (1950) to detect the glands which secrete the calcareous tubes of serpulid polychaetes. Exactly how strictly it follows the course of calcium in invertebrates is not known.

Spooner (1949) studied the absorption of radioactive strontium and yttrium by marine algae: comparing the decay characteristics of specimens of water in which the alga had been kept with control specimens, he was able to estimate the amount of strontium, as apart from yttrium, which was taken up by the weed. He suggests that the extraction of yttrium from the water involves adsorption by the surface of the tissue as well as ionic exchange, both processes being important with red algae. Autoradiographs of microtome sections of *Rhodymenia* have therefore been made to follow up Spooner's suggestion: they show, as he predicted, a surface adsorption layer of ions comprised mainly of yttrium, but also with some strontium. In animals, specially in a form like *Mytilus*, on which preliminary experiments were carried out, such adsorption detracts considerably from the value of quantitative work, for the isotopes are not only adsorbed on any glass surfaces involved in the experiment, but also on the shell of the bivalve, the byssus threads which may be secreted during the experiment, the faeces, and any particle of detritus or micro-organism in the sea water or on the surface of the body. The decay-curves of pieces of *Mytilus* shell which have been immersed in activated sea water, then washed in distilled water, placed on a microscope slide and presented to a GM4 counter, resemble those of similarly treated *Rhodymenia* fronds (Spooner, 1949): about 90% of the activity is due to yttrium which is differentially concentrated.

^{90}Sr, the radioactive strontium which was used for the present investigation, was supplied by the Radiochemical Centre, Amersham. It has a half-life of 25 years; it breaks down by β-particle-emission to ^{90}Y. The yttrium is itself radioactive and has the comparatively short half-life of 63·4 hr., changing in turn to stable zirconium, ^{90}Zr. Tests made at Amersham show that 1 mc. of the ^{90}Sr would have a total weight of strontium within the range 10–70 μg.; no inactive strontium was used for the extraction process, which was carried out using lead as carrier. Therefore, when sea water is activated by this ^{90}Sr to the extent of 100 μc./l. there is an addition to the water of not more than 7 μg. of strontium; the doses which were employed for marine organisms were kept within this quantity unless otherwise stated. Sea water from the English Channel contains 10 mg. Sr/l.: the addition of 7 μg. means, therefore, an increase of only 0·07%.

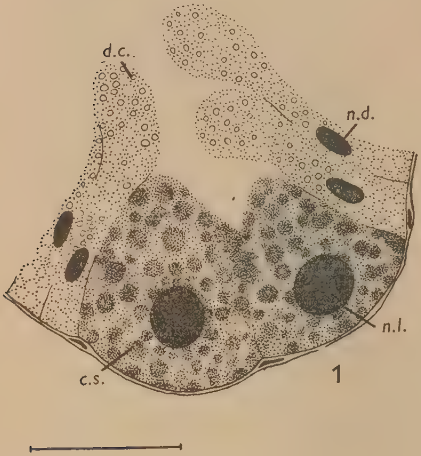
The uptake of ^{90}Sr and ^{90}Y by the tissues of certain invertebrates was studied by means of autoradiographs. These detect the distribution of comparatively minute quantities of the tracer element, and are particularly useful in studying small invertebrates which have soft bodies, since autoradiographs can be made with sections of the intact animal and the concentration of the isotope by specific cells can be followed. For the preparation of such autoradiographs animals were fixed in absolute alcohol—a fixative which is useless for the preservation of cytological detail but advantageous in that, unlike acid fixatives, it does not appear to inhibit the sensitive emulsion of the stripping film in recording tracks of the β-particles which are emitted during the decay of the tracer. After fixation the tissues are cleared in xylol, embedded in paraffin wax, and sectioned to a thickness of not more than 10 μ. Sections are floated on to slides which have been previously coated with 1% gelatine solution; later the wax is removed with xylol, the tissues hydrated, washed well in distilled water and covered with the fine-grain stripping film supplied by Kodak Ltd. The sensitive emulsion of the film is thus brought into intimate contact with the tissues, and this exposure may last from a few days to several weeks; during such a period the slides are stored in a light-tight box in a refrigerator. At the appropriate time the film, still covering the sections, is developed in amidol developer; the slides may then be placed in haemalum to stain the tissues, and the stain differentiated with 1 % HCl, which, at the same time, removes it from the film stripping.

The use of ^{90}Sr concerns two tracer elements, strontium and its daughter-product yttrium; it would be an advantage to have some method by which the activity derived from the strontium intake alone might be shown on an autoradiograph. Since ^{90}Y has a half-life of only 63·4 hr., over 99 % of any quantity of this element will decay in 19 days. When an organism takes into its body both ^{90}Sr and ^{90}Y the activity due to this yttrium may be neglected if sections of the tissues be set aside for 19 days before they are covered with photographic film. The resulting autoradiographs can be compared with those made from adjacent sections of the same animal which were covered with film a few hours after the tissues were fixed: the latter will show as the nuclear track in the emulsion of the film the position of β-particles from both the ^{90}Sr and ^{90}Y.

UPTAKE OF ^{90}SR BY *ARION HORTENSIS* AS COMPARED WITH THAT BY AN OPISTHOBRANCH

The land pulmonate stores in the tissues of its body calcium salts which may be used for the growth and repair of the shell and perhaps in buffering the gut (Manigault, 1939; Robertson, 1941). The lime cell of the digestive gland is the main storage place; also around blood vessels in both slug and snail are cells filled with calcium spherules, and in the mantle are the glands which secrete the shell (Prenant, 1924; Barr, 1928). These terrestrial molluscs obtain all their calcium from food: this mineral element, one of the most important in their metabolism, is preserved in the tissues in large quantities when it is available; in contrast, marine molluscs have a ready supply of calcium ions at hand in the surrounding medium and their reserves may be relatively low. In order to see whether the divalent ions of strontium follow a distribution which is similar to that of calcium in the body of a terrestrial, soft-bodied animal there is some advantage in using the slug or snail: they feed readily in captivity, and their calcium metabolism has already attracted the attention of many workers so that the distribution of the element within the tissues is well known.

Arion hortensis was fed with lettuce on which had been evaporated a solution of ^{90}Sr: the average consumption of isotope was of the order of 4 μc./g. of body weight, though a considerable amount did not enter the tissues, but was lost in the faeces. The slugs were given from one to four meals, each at night; they were fixed immediately after eating the contaminated food, or taken from this and put on uncontaminated leaves for a day or two before being fixed. Autoradiographs of the tissues of an individual which has been given only one meal with ^{90}Sr, and fixed immediately after it, show the isotope in both types of cell of the digestive gland—the digestive cell and the lime cell, more in the former. It would appear that both take up the ions directly from the lumen of the gland and they are scattered in the cytoplasm. Such absorption by the digestive cell is well known; but no investigation has proved that the lime cell gets its calcium by this route, yet if strontium enters thus, as well as ^{32}P (Fretter, 1952), it is highly probable that calcium would do likewise.



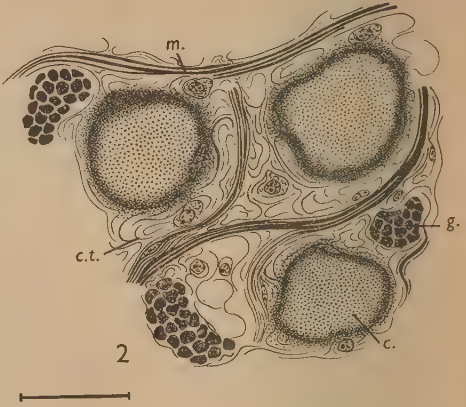


Fig. 1. *Arion hortensis.* Part of a transverse section through the digestive gland to show the distribution of ^{90}Sr. Stippling represents the superimposed autoradiograph. Fixed 3 days after commencing to feed on lettuce on which had been evaporated a solution of ^{90}Sr. *c.s.*, calcium spherule; *d.c.*, digestive cell; *n.d.*, nucleus of digestive cell; *n.l.*, nucleus of lime cell. Scale, 30 μ.

Fig. 2. *Acanthodoris pilosa.* Part of a transverse section through the periphery of the mantle to show the accumulation of ^{90}Sr. The specimen had been in activated, filtered sea water for 17 hr. and then in normal sea water for 12 hr. Stippling represents the superimposed autoradiograph. *c.*, calcium concretion; *c.t.*, connective tissue; *g.*, gland cell; *m.*, muscle fibre. Scale, 40 μ.

Slugs which have eaten larger quantities of the tracer over a period of 3 days show, in autoradiographs of their tissues, localization of strontium ions around the calcium spherules which fill the lime cells (Fig. 1, *c.s.*), and a general scattering in the rest of the cytoplasm of these cells; similar individuals, which have been kept on uncontaminated lettuce for several days before fixation, show that elsewhere in the digestive gland little of the isotope occurs for it is concentrated for storage in the one type of cell. Ions would appear to enter this cell from the haemocoel—into which the tracer passes from the digestive cell (*d.c.*)—as well as from the lumen of the gland; the two routes have already been described for the entry of ^{32}P (Fretter, 1952). The digestive gland offers the largest, yet not the only area of absorption from the gut: the intestinal epithelium is also permeable to ions. The slug, which feeds at night, retains undigested matter in the intestine during the following day. On its slow course towards the anus this food loses ions which pass into the surrounding epithelium; they are not stored here, but are lost directly to the haemocoel. The anterior aorta traverses the haemocoel and is surrounded by a reserve layer of calcium to which passes some of the strontium: a slug which has been starved for a week after feeding on the isotope shows that the element is still retained here. The third type of calcium cell, that in the mantle, may contain the tracer within 12 hr. after its entry into the gut; these cells take the isotope directly from the blood.

The few experiments with *Arion* reveal that strontium, which is in the same group in the periodic table as calcium, though an unlikely constituent of the natural diet, is taken up by the gut and concentrated in the calcium stores. It is very probable, however, that there are quantitative differences between the uptake of the two elements by the storage tissues. Now in a marine animal, divalent cations may enter the tissues with the food, by way of the gut, or, as Bethe (1934) has shown for *Aplysia*, Ca^{2+} and Mg^{2+} may penetrate the integument from the surrounding water. Bethe's work was not extended to strontium which, present only in traces in sea water, is of much less importance. However, if a small specimen of *Aplysia punctata*, in which the buccal cavity is carefully ligatured without damage to the surrounding tissues, be placed in activated water for 8 hr., autoradiographs of the whole animal show the passage of strontium ions through the general surface of the body; the gill with its thin wall and active transport systems displays in its tissues the greatest activity.

A comparatively rapid accumulation of strontium from the surrounding water is seen in the nudibranch *Acanthodoris pilosa*. It is well known that dorids may have an unusually high amount of mineral matter which is concentrated in the mantle. An examination of the composition of the body tissues of *Archidoris britannica*, after removal of mucus and visceral mass, shows that calcium comprises 2% fresh weight, magnesium 0·93%, and strontium 0·08% (McCance and Masters, 1937); this proportion of strontium to calcium is higher than in sea water. In *Acanthodoris*, as in *Archidoris*, the mineral deposits scattered through the mantle give solidity to the thick covering of the body. McCance & Masters suggest that such nudibranchs, which in the course of evolution have lost the shell, never acquired the necessary excretory mechanism for dealing with ingested calcium and consequently accumulate it. If a specimen of *Acanthodoris* be placed in sea water which has been passed through a Berkefeld filter (KF grade) and activated with 90 μc. ^{90}Sr/l., autoradiographs of the tissues show that, after 10 hr., strontium ions which have passed into the body are most numerous in the mantle, gathering around the calcium concretions there; no such concentrations occur elsewhere (Fig. 2, *c*.). Some ions, though relatively few, are in the lumen of the gut. This, as well as the outer surface of the body, may be the route by which they have entered. An individual which has been in the activated water for 36 hr., and then in normal sea water for a day, shows the strontium localized around the mineral concretions and in the cells which surround these (Fig. 4B). Some ions are leaving the body with waste matter from cells of the digestive gland. The radioactive strontium which has gathered in the mantle represents an exceedingly small amount, yet it indicates that in the nudibranch the mineral ions may be derived directly from the water, as well as by way of the food.

UPTAKE AND EXCRETION OF ^{90}SR BY *MYTILUS EDULIS*

Marine lamellibranchs have attracted the attention of more scientific workers than any other group of molluscs, yet many problems of their metabolism are imperfectly understood: some of these concern the growth of the calcareous shell. This growth continues during periods of starvation and, since there is little storage of calcium in the tissues, points to the uptake of salts directly from the water. If uptake occurs it would appear to be to no slight degree: Chatin & Muntz (1895) calculated that 75 g. of shell formed by an oyster in 12 months correspond to about 27 g. calcium; this means an absorption of 0·52 g. per week. Orton (1935) puts the weekly absorption rate at about a third of this, and he states (1925) that the shells continue to grow in the absence of food. Later Galtsoff (1934), in studying the Virginia oyster, finds that from November to the end of April, when these animals are for the greater part of the time in a state of hibernation and have no food in the digestive tract, the weight of the soft tissues remains practically stationary whilst that of the shell increases at about the same rate as during the months of feeding. Such statements indicate that calcium from the water is taken up directly into the tissues of the bivalve: yet Robertson (1941) failed to detect direct uptake of calcium ions from sea water in certain common gastropods and lamellibranchs. It may be that the methods he employed were not sufficiently sensitive to detect minute quantities.

Fox & Coe (1943), in describing the biology of the Californian sea mussel (*Mytilus californianus*), state that an individual measuring 100 mm. in length accumulates in its tissues and shell 17 g. calcium in a year; the calcium is not available in such quantities in the food, and must, they say, be obtained from the water in particulate or soluble form. If the tissues are permeable to ions such a quantity of calcium can be readily obtained: Fox, Sverdrup & Cunningham (1937) have shown that a medium-sized mussel may pass water through its gill chambers at an average rate of 22,000 l. a year, provided filtration continues incessantly, and this volume of water would contain about 9·2 kg. of calcium as salts in solution or suspension; an individual need utilize only a very small quantity of this to obtain the required 17 g. Yet if the calcium in artificial sea water be cut down to one-eighth its normal value the organic matter of the newly formed shell of *Pedalion* is deposited in the usual way (Bevelander & Benzer, 1948), but there is no calcification—the animals in this experiment may be influenced by factors other than the low calcium content of the artificial medium.

Korringa (1952) suggests that the oyster collects calcium ions from the sea in much the same way as it extracts and accumulates other positive polyvalent ions such as Fe, Cu, Zn, Fe, Hg and Mn, which are present in sea water only in small amounts: ions, if adherent to the mucous feeding sheets would be automatically collected and ingested. The use of autoradiographs presents an opportunity of finding whether this occurs, and may indicate which tissues accumulate specific ions. From results obtained by this method Bevelander (1952) concludes that both fresh-water and marine molluscs take up labelled calcium and phosphate ions. The experiments on which these conclusions are based concern only lamellibranchs: mantles and shells were removed and autographs made only of these parts; there was, apparently, no attempt to trace the course of the ions or their localization in any other part of the body.

Uptake

Small *Mytilus* measuring 1·0–1·5 cm. in length were chosen for the present study so that autoradiographs could be made of the entire body; most results were then verified on larger specimens. The activity of the sea water in which the experimental animals were placed varied from 30 to 100 μc./l.; sometimes this water contained a culture of dinoflagellates, at other times it had been passed through a Berkefeld filter. Doses up to 450 μc./l. did not appear to damage the tissues. Much of the yttrium and some strontium would be adsorbed on the vessel in which the animal was kept, and on its shell. The higher doses give an insight into the way in which the mussel may deal successfully with an unusual influx of ions into the body.

When dinoflagellates are present in the activated water a larger amount of tracer enters the body of the mussel, for the flagellates concentrate the ions to some extent and are readily collected by the feeding currents. It is seen from autoradiographs that after *Mytilus* has been in water with these flagellates for 2½ hr. the stomach displays the area of greatest radioactivity, and the tracer has passed to the digestive gland where strontium and yttrium ions are most numerous in the distal part of the cytoplasm of the digestive cells, and are scattered proximally. Some activated food has by this time entered the intestine, and from it ions of both ^{90}Sr and ^{90}Y penetrate the epithelium of the intestine and enter the haemocoel. Absorption of ferric saccharate by the intestine of a marine mollusc has been described by Gabe & Prenant (1949) for chitons, though these authors do not mention the fate of the iron which is taken up in this way. Some preliminary work with autoradiographs of *Lepidochitona cinereus* and *Patella vulgata* shows that the intestinal epithelium in both of these molluscs is permeable to strontium ions which pass by this route to the haemocoel. The long intestine in the herbivore is known to be concerned with the elaboration of faeces (Graham, 1932) and its permeability to ions may be of some importance; the food is slow to pass along it and the faeces are of considerable bulk. Yonge (1926*a*), working with *Ostrea edulis*, finds no evidence of absorption in the epithelium of the gut. He is not, however, concerned with passage of ions. It is easy to imagine that an epithelium may act as an area of ion absorption or exchange, yet be impermeable to the droplets of oil, blood corpuscles, or small diatoms which Yonge employed and which he finds are taken up by cells of the digestive gland and by phagocytes only. In *Mytilus* some strontium ions, though fewer, pass through the wall of the stomach.

In experiments with filter-feeders it is difficult to prevent the entry of particulate matter into the gut. Even if the shell of *Mytilus* be thoroughly cleaned and the animal kept in Berkefeld-filtered water (KN grade of filter) 3 days to clear the larger particles of detritus from the mantle cavity before the experiment, the results may occasionally show such particles in the gut. These preparations were made for the experiments which are cited below. MacGinitie (1945) has shown that in *Urechis* (Echiuroidea) particles between 36 and 90 Å. in diameter may be caught by the mucous feeding sheets and with them enter the alimentary canal; such food may form an important part of the diet of a microphagous feeder. In order to prevent entrapped particles from entering the mouth of *Mytilus* attempts have been made to block the oral opening without injury to any part of the body, but these did not meet with complete success.

If small *Mytilus* be placed in filtered sea water activated with 100 μc. ^{90}Sr/l. autoradiographs of the sectioned tissues show that after 2 hr. strontium ions are present in the lumen of the gut, in the digestive gland, and in the tissues of the gill filaments. Their occurrence elsewhere in the body will be neglected for the present. In some specimens ions may be adherent to the outer surfaces of the gill filaments and labial palps, also to secretion which may be in contact with these surfaces. The amount of tracer which has entered the body of an individual is greater than could have been admitted into the alimentary canal with occasional particles in the filtered sea water: tracer ions have been taken up directly into the tissues. Their entry may be by one of two routes, or by both of these. The extremely large surface presented by the single pair of ctenidia of a lamellibranch may offer an area for ionic exchange or absorption, even though, as Yonge (1928) has shown for *Ostrea*, the surface is impermeable to molecules of glucose. Ronkin (1950), using excised fragments of the gills of *Mytilus edulis*, traced a slow penetration of ^{32}P into the tissues from the artificial sea water with which they were irrigated: chemical and radioactive assay revealed that only 0·06% of the intracellular phosphate was exchanged after the tissues had been exposed for 140 min. Such an experiment cannot refer to uptake by the outer surface of the epithelium alone, since the cut ends of the filaments were exposed to the external medium and this might penetrate through the open blood spaces. However, Ronkin assumes that ionic exchange takes place at the surface of the ctenidium, and most of this, he suggests, is for use in that organ. The filaments receive their blood from the kidney and other capillaries: their blood pressure is low and the flow through them too slow to serve as an efficient transport mechanism from their tissues to other parts. It is apparent from autoradiographs that strontium ions enter the body by this route. If a medium-sized *Mytilus* has the shell wedged open slightly to give free access to the mantle cavity, and is placed so that its posterior half is in activated sea water, after 40 min. some strontium ions are within the ciliated cells of the gill filaments. Since no activity can be traced within the gut, and since in so short a time it is unlikely that ions, which might have entered by this route, would have circulated in the blood stream and be picked out almost specifically by these cells, it is assumed that the strontium has made direct entry to the ciliated epithelium of the gills. However, such entry of ions into the body would not appear to be the major one. The primary function of the gills is feeding, and ions which adhere to the mucus secreted by them may automatically find entry to the mouth. Korringa (1952) thinks it possible that the electrical properties of both food particle and feeding sheet may determine whether or not particles are readily caught by a bivalve: the oyster takes up into its body the positive polyvalent ions which have been previously mentioned, though positive monovalent ions like Na^+ and K^+, and negatively changed ions, are not easily caught. If this be so then strontium ions should be readily collected. No *Mytilus* which has been sectioned shows the mucous feeding sheets; these, if present, were destroyed in the processing for autoradiographs. In occasional individuals secretion on the ctenidial surface and on the labial palps is present, and to this some ions adhere. *Mytilus* which have been left for varying periods—a few hours to 3 days—in filtered sea water activated with ^{90}Sr all suggest that the gut is the more important area for ingress of ions to the tissues; presumably they have been directed there by the feeding currents. Their concentration is greatest in the digestive gland.

If strontium enters so readily the tissues of the gills, and, more especially, the gut, there is no reason for believing that the divalent ions of calcium would not follow a similar course and so provide for the continuous growth of the shell even in starved individuals.

The digestive gland of lamellibranchs consists of one type of cell (Yonge, 1926*b*) which is concerned with ingestion and digestion. Strontium and yttrium ions which pass into the gland in *Mytilus* are not accumulated for storage in these cells: they enter the haemocoel and are circulated to all tissues of the body. Bevelander (1952) in his discussion on calcification in molluscs states that 'calcium ions present in the water are ingested by the organism and are localized in several regions'. Of these regions he mentions only the mantle saying that the localization there is essentially the same in both freshwater and marine molluscs and he figures an autoradiograph of *Anodonta grandis* with tracer calcium concentrated in the periphery, slightly below the epithelium and in the epithelial cells themselves. In a similar position Trueman (1942) finds scattered calcium stores in *Tellina tenuis*. If strontium follows the path of calcium one might expect to find in experimental animals an accumulation of ions in these same parts which differentially select material for the production of the shell. Small *Mytilus* which have been in activated sea water up to 5 days and then in normal sea water for periods ranging from a few hours to 3 days show only a scattering of strontium in these mantle tissues and no pronounced localization. It may be that the experiments were carried on for too short a time or, rather, that the more abundant unlabelled calcium ions are preferentially selected. Actually in the mantle of *Mytilus* there is no heavy storage of calcium which might accumulate the divalent cations as a reserve comparable to that in the liver of a slug. If crystal filaments of shell be accidentally autographed with the soft tissues, however, a layer of adsorbed strontium ions is seen to encircle each. Normally strontium, if present in the lamellibranch shell, is only in very small amounts: Trueman (1942) has shown that in *Tellina tenuis*, *T. baltica* and *Donax vittatus* the shell contains traces of strontium which he suggests may contribute towards the formation of aragonite.

Excretion

Perhaps the most surprising results from the present experiments concern the rapidity with which the ions of both strontium and yttrium are accumulated for excretion, bearing in mind that the strontium, as displayed in autoradiographs, is an indication of the events of not more than 0·07% of the total quantity present in the sea water for concentrations of 100 μc./l. of water. Presumably a much greater percentage of unlabelled ions are following a similar course through the tissues of the mussel.

The excretory system of lamellibranchs consists of two separate groups of organs, the kidneys and the pericardial glands; to these may be added the digestive gland which, though with other major functions to perform, liberates to the intestine waste from its own metabolic activities. Takatsuki (1934) found that in *Ostrea edulis* these organs work in conjunction with amoebocytes which free the body of foreign or indigestible particles: they ingest these and remove them by way of the excretory organ, pericardium, surface of the auricle, rectum and mantle cavity; no such particles are taken up directly by cells of the excretory tubules. *Mytilus* which have been in activated water for 10 hr. show that cells of the pericardial gland are accumulating the tracer elements (Fig. 3A). These glands (*p.g.*) surround the auricles (*a.*) and so come into contact with the blood from which the ions are extracted; there is no indication that they have entered the cells of the gland exclusively, or even to any appreciable extent, by means of amoebocytes transporting them there as described for the course of waste particulate matter in *Ostrea* (Takatsuki, 1934). As more tracer enters the body of the mussel, with longer periods of exposure to the activated water, so a heavier accumulation of ^{90}Sr and ^{90}Y is found in the pericardial gland. Cuénot (1899) states that the phagocytic cells of the glands which capture particles may eventually transport them from the

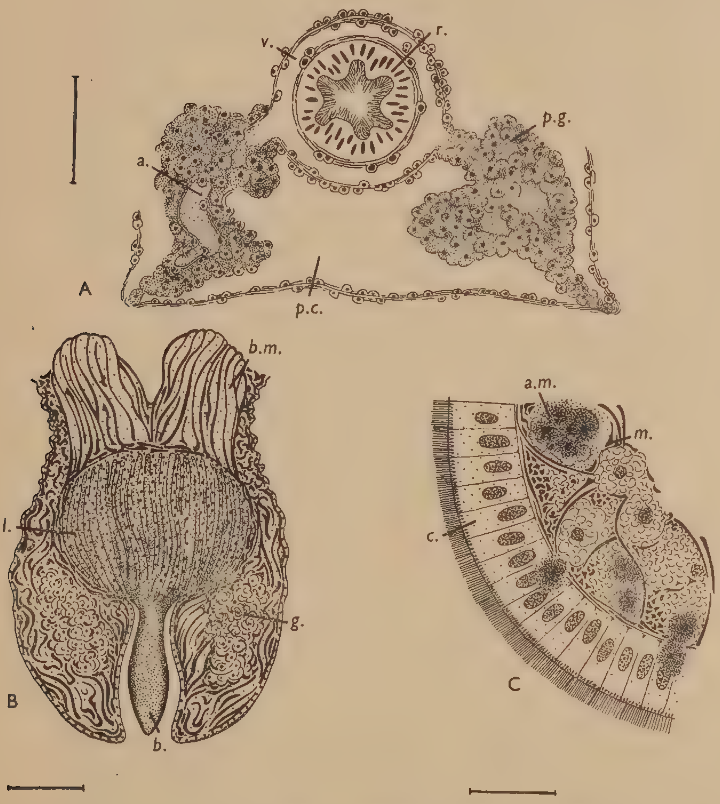


Fig. 3. *Mytilus edulis.* A, transverse section through the heart and pericardial glands. Stippling represents the superimposed autoradiograph. The mussel had been in activated sea water for 10 hr. Scale, $250\,\mu$. B, transverse section through the foot, after the mussel had been subjected to a high dose of ^{90}Sr. Scale, $500\,\mu$. C, part of a transverse section through a labial palp, after a high dose of ^{90}Sr. Scale, $25\,\mu$. Stippling represents the superimposed autoradiograph. *a*, auricle; *a.m.*, amoebocyte; *b.*, byssus thread; *b.m.*, byssus retractor muscle; *c.*, ciliated epithelium; *g.*, byssus gland in foot; *l.*, glandular lamellae dividing byssogenous cavity; *m.*, muscle fibres; *p.c.*, pericardial cavity; *p.g.*, pericardial gland; *r.*, rectum; *v.*, ventricle.

gland: they deposit the waste in connective tissue or carry it from the body by way of the gills or palps. Alternatively, the phagocytic cells may discharge their contents directly into the pericardial cavity (Takatsuki, 1934), which is in communication with the lumen of the kidney by the renopericardial duct.

The absence of the tracer elements from the kidney tissue of these experimental animals seemed somewhat surprising, and in consequence heavier doses were given to some individuals to find whether the kidney cells might then clear the blood of the ions. The doses ranged up to 450 μc./l., increasing the strontium content of the sea water by not more than 0·315% and experiments lasted up to 3 days. Yet autoradiographs revealed no accumulation by the excretory tissue. It may be that in the kidneys only the waste products of katabolism accumulate and are elaborated: ions, as ^{90}Sr and ^{90}Y, taken up from the surrounding water and not incorporated into the body tissues, and, according to Takatsuki (1934), indigestible particles which have been injected into the body, are dealt with by other means.

Mussels which had been subjected to high doses of the tracer showed its localization in the tissues of the labial palps, the gills and the byssus gland. Numbers of amoebocytes, gathered in the connective tissue and blood spaces of the palps, were laden with strontium and yttrium ions (Fig. 3C, *a.m.*): they may have migrated here from other parts of the body to discharge their contents to the exterior; only an occasional wandering cell was seen in the epithelium—if any had found their way to the surface they had been removed on fixation, or subsequently. Autoradiographs gave a similar picture of the blood spaces of the gills from which amoebocytes pass to the mantle cavity carrying excess ions. In the byssus gland the tracers were in the secreting cells, presumably extracted from the blood, and they were passed into the byssus threads (Fig. 3B, *b.*; Fig. 4A). In *Anomia* the byssus is of a peculiar form being partly calcified and of a stony consistency, for in this genus the secreting cells selectively absorb the calcium from the haemocoel. Such does not normally occur in *Mytilus*, and with the lower doses of the tracer elements there is no perceptible activity in the gland, yet this may, apparently, liberate divalent cations from the blood when their level is high.

Mention must still be made of the excretory function of the digestive gland. The tubules comprise only one type of cell (Yonge, 1926*b*) which is active in the uptake of ions from the gut. After a period of feeding yellow concretions may be found to accumulate in a distal vacuole of the cytoplasm—presumably the waste products of cellular metabolism, including digestion. Concretions are expelled to the lumen of the gland and so reach the intestine: since a large number of digestive cells may excrete at any one time the volume of this faecal matter can be quite considerable. Mussels which have been in activated, filtered sea water from 3 to 5 days and then in normal sea water for a corresponding length of time, show strontium and yttrium ions leaving the body with waste from the gland, and the tracers can be found leaving the cells with

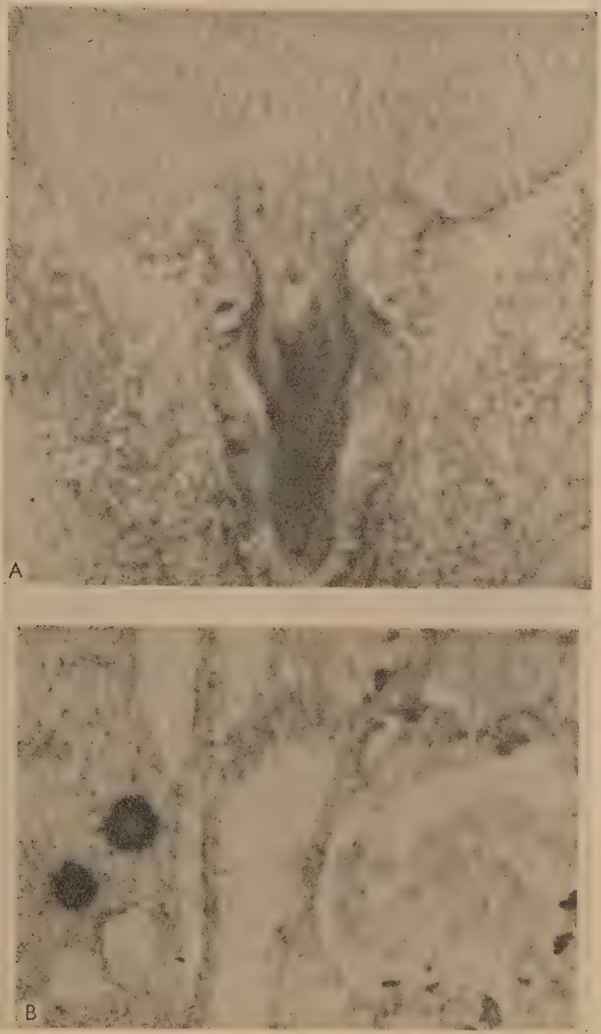


Fig. 4. A, *Mytilus edulis* subjected to a high dose of ^{90}Sr. Photomicrograph of part of a transverse section through the foot, with superimposed autoradiograph, to show ^{90}Sr and ^{90}Y in the byssus thread. B, *Acanthodoris pilosa*, photomicrograph of part of a transverse section through the mantle, kidney and hermaphrodite gland, with superimposed autoradiograph, to show ^{90}Sr localized around two mineral concretions in the mantle.

the yellow concretions. Some ions taken from the gut by the digestive cells may never leave them until they are excreted thus. This would not appear to happen to all, for there is evidence that ions which have circulated in the blood may eventually be taken back by the digestive cells to be freed from the body. Such evidence comes from animals which after taking up the tracer from filtered water are placed in normal sea water for a number of days: the digestive gland may show an accumulation of ions which is just as heavy after 5 days in normal sea water as after one, although during those 5 days excreta from the gland, carrying the tracer with it, have passed to the intestine.

WANDERING PHAGOCYTIC CELLS IN *CALYPTRAEA CHINENSIS* AND *PLATYNEREIS DUMERILI*

The importance of phagocytes in the metabolic activity of lamellibranchs has long been recognized (Cuénot, 1899; Yonge, 1926*a*, *b*). Occurring in large numbers they provide a source of digestive and excretory activity. In their cytoplasm are elaborated digestive enzymes to deal with such ingested particles as diatoms, and even blood corpuscles; their excretory function, at least in *Mytilus*, is extended to the uptake of excess ions, for which they may take the role of an emergency system. Less recognition has been given to the phagocytes of gastropods. Millott (1937) suggests that in nudibranchs they are of significance in excretion, ingesting effete matter from the haemocoel and discharging it into the lumen of the gut; he found this to be their activity with particles of iron saccharate. Prosobranchs may have phagocytes which are similarly excretory in function. If specimens of *Calyptraea* be placed in filtered sea water activated with ^{90}Sr to the extent of 100μc./l., ions pass into the body, and, circulated in the blood stream, they may be carried to all tissues. Autoradiographs of individuals which have been in activated water for 2 days show concentrations of strontium and yttrium in phagocytic cells. These may occur in various parts of the body: the tissues of the foot, the bases of the gill filaments, the wall of the pericardium and of the kidney. It is doubtful whether these positions can be connected with any nutritive function of the wandering cells. Whatever their action may be it is associated with an increase of strontium in the external medium of 0·70%: in the body tissues the increase could therefore be only slight. This suggests that the phagocytes are extremely sensitive to changes in their environment. Perhaps one of their important functions is in helping to maintain an ionic steady state within the body. The localization of ^{90}Sr is also marked in the hypobranchial gland. It elaborates large quantities of secretion, and in taking up constituents from the blood presumably abstracts the divalent cations of strontium, which are then freed from the body with its secretion.

Wandering amoebocytes occur in polychaete worms. Romieu (1923), referring to them as leucocytes, says that they may collect excretory matter from the body and transport it to the epidermis, where they may be seen in *Dodecaceria concharum*. Similar phagocytosis by leucocytes occurs in nereids and *Aphrodite aculeata* (Fordham, 1925); the cells are particularly large and active in *Perinereis cultrifera* (Romieu, 1923). In polychaetes it is recognized that one type of leucocyte may deal with waste, another with food stuffs. The former, which phagocytose and eliminate noxious material, are called lymphoidocytes; the latter, which transport and release food into the blood and tissues, are trephocytes. Liebman (1946) describes and figures the two in *Amphitrite crinata*. Since in certain molluscs amoebocytes are active in the transport of ions it was thought that the investigation of a polychaete might show that in this class also they can be similarly employed. Autoradiographs of sections of *Amphitrite gracilis* and *Platynereis dumerili* which had been subjected to the dose of 100 μc. ^{90}Sr/l. of sea water, showed no use of amoebocytes in dealing with tracer ions which have entered the tissues. *Platynereis* were then kept for 3 days with 3 times as much tracer in the water, some in filtered sea water, and others amongst small quantities of sand and detritus from the coralline pool in which they had been collected. Autoradiographs of those individuals revealed wandering cells laden with both strontium and yttrium ions (Fig. 5, *l.*). They were found most commonly beneath the ectoderm (*b.m.*) of the body wall, a position which suggests that they are lymphoidocytes ridding the body of the tracer elements. The greatest amount of radioactivity was found in a worm which had ingested a fragment of weed

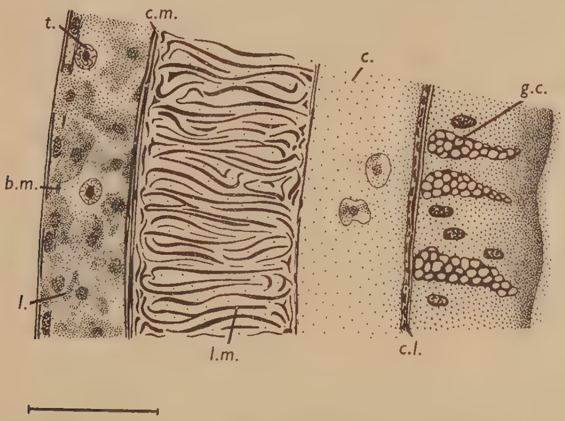


Fig. 5. *Platynereis dumerili*. Part of a transverse section. Stippling represents the superimposed autoradiograph. The specimen had been in sea water activated with ^{90}Sr for 3 days, and had taken activated food into its gut. *b.m.*, basement membrane of ectoderm; *c.*, coelom; *c.l.*, circular and longitudinal muscles of gut; *c.m.*, circular muscle of body wall; *g.c.*, gland cell in epithelium of gut; *l.*, lymphoidocyte; *l.m.*, longitudinal muscles of body-wall; *t.*, trephocyte. Scale, 250 μ.

together with some detritus; from these contents ions passed through the wall of the alimentary canal (*g.c.*) to the coelom (*c.*). The gut would appear not to be the only path by which ions enter the body, although they find entry here even in the absence of food: individuals kept in radioactive sea water for half an hour show little tracer in the alimentary canal and more in the integument, suggesting an absorption here. Lymphoidocytes with some ^{90}Sr and ^{90}Y are occasionally in the coelom: always those with the highest concentration are clustered between the basement membrane of the ectoderm and the underlying fibres of circular muscle (*c.m.*). Amongst them occurs an apparently less frequent type of cell with little or no tracer in its cytoplasm. This is presumably the trephocyte (*t.*) which is concerned with the transport of nutritive material. The subepithelial clusters of lymphoidocytes may become very pronounced if a worm be subjected to high doses of the tracer for several days. Some of these cells, always with the tracer ions, have been found between the cells of the ectoderm and on its outer surface, behaving in a fashion which is similar to the wandering cells of *Mytilus.*

This work was carried out at the Plymouth Laboratory of the Marine Biological Association, and was aided by a grant from the Browne Research Fund of the Royal Society. I wish to express my thanks to many members of the staff of the Plymouth Laboratory for their help, and to the Committee of the Browne Research Fund. I am also indebted to Mr F. C. Padley of Reading University for taking photographs.

SUMMARY

If *Arion hortensis* be fed on a diet which contains ^{90}Sr, autoradiographs show that the isotope is taken up by the digestive and lime cells of the digestive gland. From the former it passes to the haemocoel; in the lime cells it is concentrated around the calcium spherules. Some of the tracer enters the body through the wall of the intestine. Calcium stores which surround blood vessels and calcium cells in the mantle also concentrate the tracer.

In *Aplysia punctata* ^{90}Sr from the surrounding water passes through the surface of the body, and especially the gill; in *Acanthodoris pilosa* ions which enter the tissues from the sea water accumulate around the numerous calcium concretions in the mantle. These marine molluscs obtain cations directly from the water as well as by way of the food.

There is a slow uptake of strontium ions by the ctenidia of *Mytilus edulis*, though, even in filtered sea water, the gut is the more important area for their ingress to the body. It is possible that they enter with the mucous feeding-sheets. They pass readily into the cells of the digestive gland. Some of the isotope taken in with the food is absorbed by the wall of the intestine; this also occurs in *Patella vulgata*, in which the intestine provides a much larger area, and in *Lepidochitona cinereus*.

Mytilus placed in filtered sea water which is activated with ^{90}Sr, so increasing the strontium content by 0·07%, show the tracer localized for excretion within 10 hr. Ions are aggregated in the pericardial glands, not in the kidney. If the strontium content of the water in which *Mytilus* are kept be increased by the tracer up to 0·315%, within 3 days amoebocytes with the element, and with its daughter product yttrium, are gathered in the connective tissue of the labial palps, and in blood spaces of the gills from which they pass to the mantle cavity. The tracers also leave the body with secretion from the byssus gland, but are not accumulated by the cells of the kidney. There is no storage of ^{90}Sr by the digestive gland which excretes the ions into the gut, nor are there high concentrations retained by tissues of the mantle.

Amoebocytes of *Calyptraea chinensis* accumulate the tracer element which enters the tissues from filtered sea water. One of their important functions may be in helping to maintain an ionic steady state within the body.

Two types of wandering cells occur in *Platynereis dumerili*: one of these, the lymphoidocyte concentrates the isotope in its cytoplasm and transports it to the outer surface of the body.

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J. Mar. biol. Ass. U.K. (1953) 32, 385–47.
Printed in Great Britain

STUDIES ON THE INTERRELATIONSHIPS OF ZOOPLANKTON AND PHYTOPLANKTON

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(Plate I and Text-figs. 1–4)

CONTENTS

	PAGE
Introduction	385
Apparatus and methods	388
Horizontal experiments	388
Vertical experiments	390
Culture of phytoplankton	391
Exploratory toxicity experiments (with results)	392
Treatment of the results	395
Experimental results	396
The horizontal apparatus	396
The vertical apparatus	413
Underwater observations	426
General discussion	428
Experiments in the horizontal apparatus	428
Experiments in the vertical apparatus	431
The size of plankton concentrations	432
The density of phytoplankton concentrations	434
Conclusions	435
Summary	442
References	443
Appendix I. List of phytoplankton organisms used	446
Appendix II. List of zooplankton organisms used	447

INTRODUCTION

It has in the past been thought that the marine plankton is more or less uniformly distributed. With more and more intensive sampling and observation, however, it has become apparent that the distribution of all forms of both animal and plant plankton is uneven in the extreme. Further, it has been demonstrated that generally, but by no means always, there is an inverse relationship between the quantities of zooplankton and phytoplankton taken in any one limited area. Attention was first strongly focused on this fact by Hardy (Hardy & Gunther, 1935), although earlier authors had remarked on its occurrence. Almost simultaneously, at that time, two hypotheses were put forward to account for this phenomenon.

Harvey (1934, and more fully in the joint paper of Harvey, Cooper, Lebour & Russell, 1935), recorded occurrences of the inverse relationship in the English Channel and proposed that grazing by the zooplankton could adequately account for the observations. His hypothesis suggests that, in areas of high zooplankton concentrations, most of the phytoplankton would be grazed down; while in areas of low zooplankton concentration it would flourish. The effect of this would be to produce the observed inverse relationship.

Hardy (*loc. cit.*) showed how his own Antarctic results substantiated earlier records by Pearcey, Castracane and others of the inverse relationship, and proposed the hypothesis of 'animal exclusion' to explain it. This hypothesis supposes that high concentrations of phytoplankton are in some way 'distasteful' to plankton animals, which, during their diurnal vertical migrations, either refrain from coming up or come up for a much shorter time in areas rich in phytoplankton. The differing speeds and directions of currents at various levels then automatically segregate the concentrations of animals and plants and produce the observed inverse geographical distribution.

Both authors acknowledge that their hypotheses are not mutually exclusive and agree that either may be operative at any one time depending upon the circumstances. The possibility that a pattern may first be imposed upon the phytoplankton by grazing and may then be maintained by exclusion of the animals from the resulting areas of increasing phytoplankton density is mentioned by Hardy (p. 310).

The conception of a direct 'lethal' effect of phytoplankton concentrations upon zooplankton has been developed particularly by Lucas (1936, 1947). Such an effect could result in an exclusion without the mediation of vertical migration and could account for those occurrences of the inverse relationship in shallower waters where phytoplankton concentrations may reach right to the bottom, as in the North Sea. There is now considerable evidence (most usefully collated by Brongersma-Sanders, 1948) on the toxic properties of blooms of coloured water, but it seems likely that this is a specialized phenomenon restricted to certain species and localities. The possibility of a less marked, but nevertheless adequately operative lethal effect amongst other members of the phytoplankton, must, however, be borne in mind. Lucas (1949) has in fact drawn together much evidence on the importance of the external secretions of both animals and plants in the communal life of many organisms, and now looks upon 'animal exclusion' as one manifestation of a widespread system of ecological inter-relationships, which embraces both beneficial and harmful effects on the part of various reactants.

Steemann Nielsen (1937), while not minimizing the possible effect of grazing, maintains that the naturally different rates of development of phytoplankton and zooplankton populations are sufficient to account for the inverse relationships. Under favourable conditions a phytoplankton maximum can develop rapidly while a zooplankton maximum must necessarily lag behind. This and the frequent occurrence of phytoplankton concentrations in areas of up-welling water, which generally has a low zooplankton stock, combine to make phytoplankton and zooplankton concentrations occur in different regions.

There is yet little evidence unequivocally in support of any of these hypotheses. Mathematical work by Fleming (1939) and observations on rates of feeding by Fuller (1937) and Gauld (1951), among others, make it clear that Harvey's idea of grazing is well within the realms of probability. Lucas's (1936) work on zooplankton distribution in partially shaded diatom cultures, and Hardy & Paton's (1947) work have not produced the looked for evidence in support of the exclusion hypothesis.

Any attempt to resolve between the ideas of grazing and exclusion, or to determine which may be the more important, must necessarily include an experimental approach. The present steady accumulation of data on distribution can only substantiate the existence of the inverse relationship, or at the very best serve as an indication of the possible means through which it arose. A most intensive survey would be needed to provide a solution to the problem in the sea itself. Essentially the problem is that of the behaviour of zooplankton in the presence and in the absence of phytoplankton. If the whole phenomenon arises through mechanical means (Harvey or Steemann Nielsen), then there need not be special behavioural characteristics, while if it arises through an exclusion mechanism it should be possible to demonstrate, in the laboratory, those patterns of behaviour which result in the observed distributions.

Lucas (1936) interprets his results as showing an avoidance by animals of illuminated, active, phytoplankton. Elimination of the confusing effects of shading in this work is made possible by the technique of producing a gradient of phytoplankton in a long tube (Bainbridge, 1949). If animals introduced into this were to show any avoidance of high phytoplankton concentrations, then support for Hardy's hypothesis would be obtained, while if they fed blindly, remaining in even the highest concentrations of phytoplankton, then Harvey's hypothesis would be supported.

Hardy's hypothesis utilizes only the powers of vertical migration of zooplankton combined with water movements, and it would therefore be most desirable to investigate the movement of zooplankton in vertical gradients. The practical difficulties of preparing stable vertical gradients and of introducing animals into them are, however, considerable and this method of approach was rejected. Instead, the vertical migrational behaviour of animals in the presence and absence of phytoplankton in simple vertical tubes was studied. It is generally maintained that members of the plankton possess negligible powers of horizontal migration and make extensive journeys only in the vertical plane.[1] I believe this view to be erroneous and am led by experimental data and observation to the conclusion that those animals now broadly grouped as planktonic fall naturally into two classes: one comprising most of the copepods, coelenterates, smaller and early larval stages, etc., which, if migrating at all, in fact only migrate vertically; and the other comprising some large copepods, the euphausids, mysids and cumaceans and larger larvae, etc., which, besides migrating vertically, can, and under suitable stimuli do, make horizontal migrations extensive enough to be of great consequence in the ordering of distribution. The experiments here described cater for these two classes of animals, the vertical tubes for the former and the horizontal tubes for the latter, while the results obtained serve to support the reality of this grouping.

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[1] The term 'zooplankton' is a very broad one covering what is not in fact a distinct group but rather a loose assemblage of animals with clearly graded powers of locomotion. Recent work (Hardy & Bainbridge, 1951) has shown that many of these animals can in fact swim much faster and for more sustained periods than has previously been thought. It is known that almost all perform great vertical migrations but any horizontal component there may be in their movements is generally thought to be random and undirected.

I am deeply indebted to Professor A. C. Hardy, F.R.S., for his constant interest in this work and for having allowed me to do part of it while employed by him with a grant from the Leverhulme Trustees; to the Royal Society whose generous grant from the Browne Fund allowed it to be completed; to the Directors of the Plymouth and Millport Laboratories who most kindly provided facilities; to Messrs Siebe, Gorman and Co., who made a loan of diving equipment; and to Dr T. J. Hart and Dr C. E. Lucas for their helpful criticisms of the manuscript. Finally, I have been encouraged and advised by many friends, to all of whom I am most grateful. Most of the work described has been approved by the University of London for award of the degree of Ph.D.

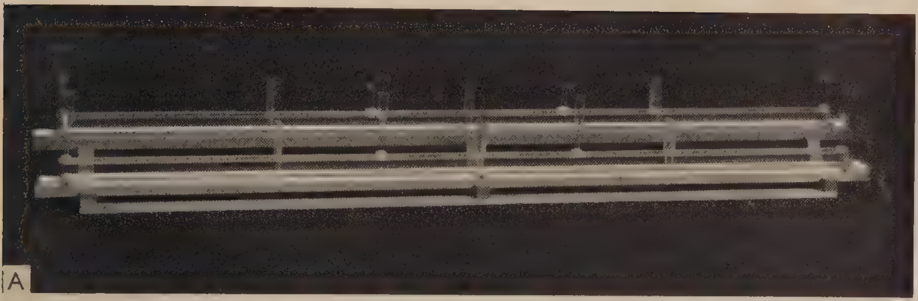
APPARATUS AND METHODS

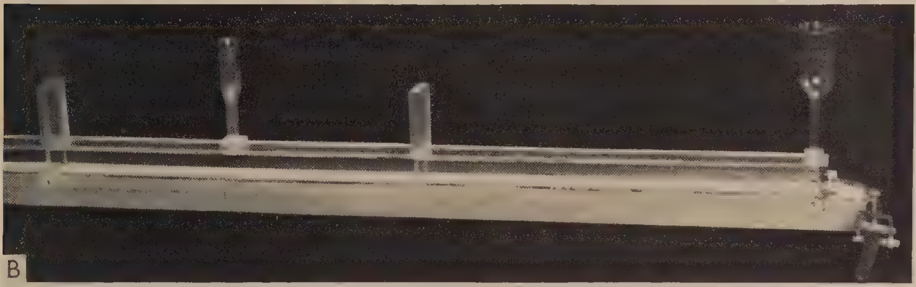
Horizontal Experiments

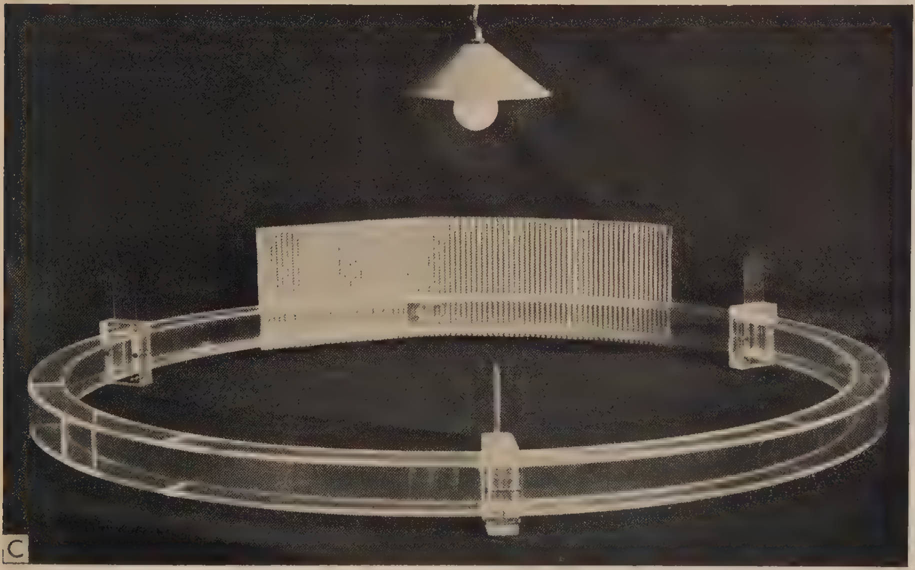
Straight Apparatus

Preliminary horizontal experiments were performed in the glass apparatus described in Bainbridge (1949). This apparatus was eventually replaced by one constructed entirely of Perspex. With this material it was possible to build up a tube of rectangular cross-section, thus eliminating those optical effects of a cylindrical tube which make the counting of small animals difficult and inaccurate. The second apparatus consists (Pl. I A, B) of a horizontal Perspex tube 48 in. long of internal dimensions 1 in. wide by $\frac{3}{4}$ in. deep. This is divisible into four equal water-tight compartments by sliding doors; funnels at the outer ends of the outer two compartments and at the centres of the two central ones allow for filling and the introduction of animals. Two of these pieces were made so that control or replicate experiments could be performed simultaneously.

In a typical experiment the central door is first closed and the apparatus filled: freshly taken ordinary sea water (hereafter referred to as 'normal'), or filtered sea water, being put into one side and water enriched with phytoplankton into the other. After a pause of 3 or 4 minutes to allow currents to cease, the central door is opened. Diffusion and mixing of the phytoplankton and normal water follows fairly quickly and within 5 minutes a visible







A. General view of second Perspex horizontal apparatus.

B. Detail of right-hand half of one tube of above.

C. Perspex horizontal circular apparatus, showing the three sliding doors, one section of a surrounding cardboard shield and the electric light.

gradient of phytoplankton exists in the central 10 in. or so of the tube, separating the clear water at one end from the slightly cloudy, enriched water at the other. For a satisfactory uneven distribution to be maintained it is necessary for the waters put into the two sides of the apparatus to be identical in temperature and specific gravity. Otherwise flowing occurs and results in the formation of two layers along the length of the tube. The enriched water is always of a lower specific gravity than sea water owing to the addition of a small amount of tap water during the preparation of the culture solution (see below, p. 391). Immediately before the experiment, therefore, the two lots of water to be used, after having been brought to the same temperature, are tested with a hydrometer and the specific gravity of the higher is brought down to that of the lower by the addition of drops of tap water. With the apparatus full, animals are pipetted into each side of it in equal numbers through the two central funnels. The distribution of animals throughout the length of the tube is now recorded at regular intervals. For this purpose the tube is divided into imaginary quarters delimited by the central door and the two outer doors. At the appropriate time the number of animals in each quarter is counted quickly and recorded in a table. When using the second apparatus, if a large number of quickly moving animals is involved, then all three doors are closed during the counting, which is thus carried out much more accurately. The doors are opened again to allow the experiment to continue.

Recording continues for the duration of the experiment and this varies from 1 or 2 hr. up to 3 days. During this time notes are made as to the stability of the uneven distribution in the tube and of the gradient. The simple criterion taken for the persistence of these is their continued visibility. This method is not always practicable, however, and in some instances water has to be removed from both ends by a pipette and the phytoplankton content assessed by counting. At the completion of an experiment the apparatus is emptied through the draining tube fitted at one end and is washed out. Occasionally a more thorough cleaning is required. The ends are then removed and the tube pulled through with a wad of cotton-wool on a string.

Circular Apparatus

One of the disadvantages of the straight apparatus is that animals consistently swimming in one direction come sooner or later to the end of the tube. If they have no reaction for turning and exploring in the opposite direction then they may continue swimming and butting into the end of the tube in an environment in which they would not have remained had they been able to sample that at the other end. To obviate this disadvantage some experiments were performed in a circular horizontal tube.

This apparatus (Pl. I c) consists of a Perspex tube 12 ft. long and 2 by $1\frac{1}{2}$ in. internal dimensions, built up in the form of a circle 4 ft. in diameter.

The whole is divisible into three equal sections by sliding water-tight doors, and openings 1½ by ½ in. at these three points allow for filling and the introduction of animals. The apparatus is set up horizontally in a darkened constant-temperature room and illuminated by a 25 W. electric bulb suspended symmetrically about 2 ft. above it.

In a typical experiment two of the doors are closed and the segment between them is filled with water enriched with phytoplankton. The remaining two segments are filled with normal or filtered water and, after a pause, the doors are opened. Animals are introduced in equal numbers at the three openings. For the purpose of counting the whole tube is divided into imaginary sixths, three of the boundaries coinciding with the sliding doors and three placed symmetrically between them. Starting from a fixed reference point in relation to the room the animals in successive sixths are counted quickly and the numbers recorded in a table. This is repeated at intervals for the duration of the experiment. At its completion water is drawn out at the three openings and counts of the phytoplankton made to assess the stability of the uneven distribution in the tube.

Vertical Experiments

The vertical experiments were performed in an apparatus consisting of two parallel Perspex tubes 48 in. long and 2 by 1½ in. internal dimensions. The tubes are held 2 in. apart by Perspex spacers and are closed at the top and bottom by removable Perspex covers. In the centre of each is a butterfly valve of 1 mm. thick Perspex which, in the horizontal position, divides the tube into upper and lower portions and, in the vertical position, presents only a very small obstacle to the movements of the animals. The valves are worked by levers, weights and strings.

In a typical experiment one tube is filled with normal or filtered water and the other with water enriched with phytoplankton. A number of animals are introduced into the top of each tube and the lids are bolted down. (The animals may be picked out and counted before use, when equal numbers are put into each tube; or roughly equal numbers may be poured in and, at the completion of the experiment, removed by filtration and counted at leisure.) The apparatus, when filled, is lowered to 6 in. below the surface of the water in the centre of a large (72 × 33 ft.) open tank and suspended, with the valves open, from a wooden boom. At intervals the doors are closed, the apparatus removed from the water and the number of animals in each of the upper compartments is counted and recorded in a table. The apparatus is then lowered back into the water and the doors opened. This is continued for the duration of the experiment, which may be from a few hours to several days.

Preliminary experiments were performed in this apparatus, but it soon became clear that the method had several disadvantages. First, rather small numbers swam up during the daytime owing to the high intensity of light and this made assessment of any difference between the two tubes difficult; and secondly, the animals were undoubtedly disturbed when the apparatus was taken out of the water and when wind caused it to move about. To obviate these disadvantages the later and main series of experiments was performed indoors in an aquarium tank.

The apparatus was suspended close to the glass of a 4 × 4 × 5 ft. tank with the tops of the tubes just above water-level. Lighting was oblique and chiefly from above and the intensity was a good deal less than that out of doors. The water circulating in the tank kept the apparatus at a temperature only a few degrees above that in the sea and the animals could be counted readily through the glass without any disturbance whatsoever. As a result of this it became unnecessary to close the doors, and these were removed and the apparatus split up to give two pairs of simple vertical tubes, each 2 ft. × 2 in. × 1½ in. This enabled duplicate readings to be taken in every experiment.

Culture of Phytoplankton

Almost all the phytoplankton organisms used in the experiments were grown in pure cultures. Occasionally natural sea water at the time of a rich diatom outburst was used, and occasionally natural water was concentrated by filtration and the concentrate used. The cultures were first grown in jars of sea water enriched with Miquel solutions and sterilized by heating to 70° C. after Allen & Nelson's (1910) instructions. More satisfactory results, however, were obtained by a modification of the 'Erdschreiber' technique, and in the bulk of the work only cultures grown in this manner were employed.

A soil extract was made by bringing a mixture of 500 g. of garden soil and 1 l. of tap water to the boil and allowing to simmer for 40 min. This mixture was left to stand for a few days and the supernatant liquid was decanted, brought to the boil, and again left for a few days. This process of decantation and boiling was continued until a clear sherry-coloured liquid resulted. The sea water used for growing the cultures was filtered through coarse filter-paper, sterilized by heating to 70° C., and allowed to cool. For every litre of sea water 50 ml. of the clear soil extract were brought to the boil and 0·3 g. of sodium nitrate and 0·03 g. of sodium hydrogen phosphate dissolved in the hot liquid. This hot solution was then added to the cold sea water and the whole stirred. After standing for a day the enriched sea water was ready for use as culture solution.

The cultures were grown variously in flasks and breffit jars standing in a window with a north light. Most of the diatoms were picked out from sea-water samples that had been enriched by the addition of an equal volume of culture solution and allowed to stand for a week or so. This ensured that only healthy, growing cells were taken. Dense cultures grown from a single cell or chain of cells were obtained in this manner. The author is deeply indebted to Dr Mary Parke who provided initial samples of all the flagellates used. These had themselves previously been picked out by the method described, although many had been in culture for some time.

Exploratory Toxicity Experiments (with Results)

The horizontal experiments are generally lengthy to perform and use large volumes of culture. It soon became apparent, when all the early experiments were giving positive migrations into the cultures, that if any were to be found producing a reverse 'exclusion' effect, then some quicker method must be used to give a preliminary indication of cultures that might produce this effect. A series termed the 'toxicity experiments' was therefore set up in order to test cultures before use in the horizontal experiments.

A number (generally twelve) of 50 ml. capacity jars with open tops were arranged on black paper trays. 20 ml. of the culture to be tested, either as grown or sometimes diluted with ultra-filtered water, was put into each jar and the concentration of cells assessed by counts on a haemocytometer slide. The animal chosen was *Hemimysis lamornae* and one of these was put into each jar and the whole batch covered with a glass plate. The animals were examined from time to time for a period of 3 days and notes were made of any that died, of the presence or absence of faecal pellets, food in the gut and other relevant observations. The trays stood on a bench in front of a window with a north light. No attempt was made to control the temperature.

The results of these experiments are summarized in Table 1, where it is seen that a total of sixty-eight different pure cultures of phytoplankton organisms was used. Control experiments, with normal sea water passed through a Berkefeld filter candle and containing no particulate matter, were used as a reference. The mean result for ten experiments with filtered water, each using twelve animals, is a total of 5·3 dead out of 12 at the end of 3 days. It will be seen that the great bulk of the cultures used gave a much better survival than this. Out of a total of seventy-one cultures tested (including three duplicates) twenty-four gave a complete survival and sixty-five gave five or less dead. These have all been assumed lacking in toxic properties although they may, of course, have very different nutritive values. Three cultures—Flagellate 12; *Gymnodinium* I and Flagellate One—each gave eight dead, *Chlorella stigmatophora* eleven dead, and *Gymnodinium* II twelve dead. These five have been suspected of having toxic properties of some kind.

In an attempt to demonstrate more clearly that these deaths were due to a toxic action and not to starvation, *Chlorella stigmatophora* was chosen for a series of experiments identical with those above, save that various concentrations of *Chlorella* were used. The results obtained from sixteen batches of jars (one with twenty-five jars, three with eight and the remainder with twelve jars) containing cultures ranging from 13,120 cells/mm.^{3} down to 585 cells are shown in Text-fig. 1A. The concentrations have been divided into five groups, the number of jars in each group adjusted to be the same,

TABLE 1. RESULTS OF EXPLORATORY TOXICITY EXPERIMENTS

Culture	Concentration (cells/mm.^{3})	Number out of 12 dead in 1st 24 hr.	2nd 24 hr.	3rd 24 hr.	Total
Flagellate 3	2,310	—	1	—	1
Flagellate 10	900	1	—	1	2
Flagellate 25	655	—	1	1	2
Flagellate K	3,760	—	—	5	5
Lancelot II Clone 8	200	—	—	1	1
L4 (IV) Clone 3	310	—	—	2	2
L4 (IV) Clone 6	410	1	—	—	1
Lancelot I Clone 4	905	—	—	1	1
L 4 (13/4) 2L Clone 1	1,480	—	—	—	—
L 4 (4/5) Clone 3	610	1	2	—	3
Chromulina pleiades	3,220	1	—	—	1
Chromulina pusilla	37,000	—	—	—	—
Pavlova gyrans	820	—	—	—	—
Coccolithus huxleyi	2,080	2	—	—	2
Isochrysis galbana	3,680	—	—	—	—
Dicrateria inornata	11,220	1	—	1	2
Pseudopedinella I	230	—	—	—	—
Flagellate One	**680**	**1**	**4**	**3**	**8**
Flagellate 5	2,120	1	—	—	1
Flagellate 22	8,800	—	—	1	1
Flagellate J	2,640	1	1	—	2
Flagellate A	1,600	—	—	—	—
Lancelot I Clone 1	1,760	—	1	—	1
Lancelot I Clone 5	830	—	3	—	3
Lancelot I Clone on 19/5	1,240	—	1	—	1
Lancelot II Clone 10	2,920	—	—	2	2
L 4 (13/4) 2L Clone 6	2,520	—	—	1	1
Flagellate 6	820	—	—	—	—
Flagellate 6*a*	3,840	—	—	—	—
Hemiselmis rufescens]	3,440	—	—	—	—
Flagellate 23	220	—	—	—	—
Flagellate 23*a*	36	—	—	2	2
Flagellate 14	4,320	—	—	1	1
Flagellate 14	2,860	—	1	—	1
Flagellate 16	555	—	—	—	—
Flagellate 19	420	—	—	1	1
Flagellate 20	230	1	1	—	2
Flagellate 21	135	—	—	—	—
Flagellate 29	500	—	—	—	—
Lancelot II No. 16 Clone 3	300	1	—	—	1
Lancelot II No. 16 Clone 3	250	—	—	—	—
Gross's μ flagellate	480	—	—	—	—
L4 (13/4) 2L Clone 5	255	1	—	2	3
Exuviaella baltica	100	1	1	2	4
Exuviaella I	9	1	1	1	3
Prorocentrum micans	14	—	1	1	2
Prorocentrum triestinum	62	—	—	—	—
Flagellate 12	**220**	—	—	**8**	**8**
Oxyrrhis marina, etc.	2	—	1·5	1·5	3*
Gymnodinium I	**310**	—	**1**	**5**	**6**
Gymnodinium I	**200**	**3**	**3**	**2**	**8**
Gymnodinium II	**82**	—	**9**	**3**	**12**
Massartia rotundata	28	—	—	2	2
Peridinium trochoidium	50	—	1	—	1*
Chlamydomonas III	1,000	—	—	1	1
Platymonas apiculata	11	—	—	—	—
Pyramimonas sp.	235	—	—	—	—

TABLE 1 (*continued*)

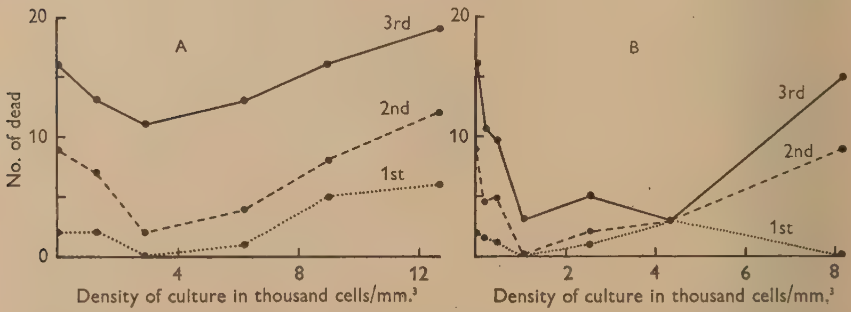
Culture	Concentration (cells/mm.^{3})	Number out of 12 dead in 1st 24 hr.	2nd 24 hr.	3rd 24 hr.	Total
Chlorella stigmatophora	**13,120**	**5**	**2**	**4**	**11**
Stichococcus sp.	570	—	—	—	—
Coscinodiscus concinnus	28†	—	—	—	—
Skeletonema costatum	35	—	—	—	—
Thalassiosira gravida	18	1	—	0·5	1·5*
Lauderia borealis	65	—	3	—	3
Ditylum brightwellii	92†	—	—	—	—
Eucampia zoodiacus	8	—	—	1	1
Chaetoceros decipiens	53	—	—	—	—
Licmophora lyngbyei	275	1	—	—	1
Naviculoid	2	—	—	1	1
Nitzschia closterium (min.)	*c.* 400	0·5	1·5	2	4*
Nitzschia closterium (normal)	73	1	—	—	1
Nitzschia seriata	12	—	—	—	—
Filtered water	—	1	8	3	12
Filtered water	—	1	—	4	5
Filtered water	—	—	2	3	5
Filtered water	—	—	2	2	4
Filtered water	—	2	3	2	7
Filtered water	—	—	—	1	1
Filtered water	—	1	1	6	8
Filtered water	—	—	6	—	6
Filtered water	—	1	—	—	1
Filtered water	—	—	2	2	4
Mean of filtered results	—	0·6	2·4	2·3	5·3

Note. The above organisms are in classificatory order as in Appendix I and not in the order of testing. Species considered toxic have been put in heavy type.

* Calculated from a total of 25.
† Cells/100 mm.^{3}.

Summary showing numbers of cultures resulting in various deaths:

No. of deaths	—	1	2	3	4	5	6	7	8	9	10	11	12
No. of cultures	24	20	12	6	2	1	1	—	3	—	—	1	1



Text-fig. 1. Results of toxicity experiments with cultures of various concentrations of (A) *Chlorella stigmatophora*, (B) *Nitzschia closterium*. Numbers of dead, out of thirty-two in A and thirty-six in B, on the 1st, 2nd and 3rd days.

and the figures expressed as numbers dead per thirty-two animals after 1, 2 and 3 days. It is seen that there is an optimum survival at a concentration of about 3000 cells/mm.^{3}. Below this the increase in deaths may be assumed due to starvation and above it to the toxic effect.

Lucas (1936) has put forward evidence for an optimum survival in *Nitzschia closterium* cultures of a certain strength, and an attempt was made to demonstrate this with *Hemimysis lamornae*. The results were treated as those for *Chlorella* and are depicted in Text-fig. 1 B. It is seen that survival is generally much better than with *Chlorella*, except at the lowest concentrations, but that again there is an optimum at a certain concentration, this time about 1000 cells/mm.^{3}. (This figure is higher than that found by Lucas who considered the optimum to lie between 25 and 100 cells/mm.^{3}, but he used *Neomysis vulgaris* and the two species may well react differently.) It may again be assumed that there is some sort of toxic effect at higher concentrations. This was not recognized in the earlier 'toxicity' series of experiments because a sufficiently high concentration of cells was not used. Such a concentration would not generally be met with in nature so the result must largely be of academic interest except, perhaps, in so far as it is an indication of what may, in lesser degree, be happening at lower concentrations and not detectable by this experimental method.

Treatment of the Results

In some of the experiments the behaviour of the animals in relation to the uneven distribution of phytoplankton in the straight or in the circular horizontal apparatus is very distinctive, but in general it is necessary to apply an appropriate statistical technique in order to determine which differences in the distribution of the animals are significant.

In the straight apparatus, for any given reading, this involves calculation of the deviation for the numbers in the left- and right-hand sides of the tube. The reading is taken to be significant if this exceeds twice the value of σ calculated for the particular number of animals in the tube. For the purpose of this work a significant difference is termed positive or negative depending upon whether it involves a movement into the phytoplankton-rich side of the apparatus or away from it.

It is not possible to apply the test for significance to more than one reading in a particular experiment and then attribute an independent and equal value to each answer, because each successive reading is to some extent dependent for its value upon those preceding it in the series. This invalidates the test used. The latter is therefore only applied to one reading in an experiment or only once to the mean of several readings. The single reading or the selection of readings averaged may be taken arbitrarily or may be chosen as typical of the experiment as a whole.

In the circular experiments the results are treated similarly. The numbers in the six sections counted cannot however be divided symmetrically about the third of the tube containing the enriched water. The number in the phytoplankton third is therefore compared with the number in the third opposite it across the circle and the numbers in the two sixths between these sectors are disregarded. This is a more rigorous test than the simple comparison of halves and in some border-line cases special counts were made of the two halves. The dividing line was then that diameter at right angles to the one passing symmetrically through the phytoplankton third. This special count was most valuable when there had been more than the normal spreading of the phytoplankton sector and parts of the disregarded sixths were rich in both phytoplankton and zooplankton.

In the vertical experiments such changes as occur in the vertical migrational behaviour in the two tubes have again to be treated mathematically in order to determine whether they differ significantly from expected chance variations. The test employed in this case is a test of independence and is applicable because the data fit a two by two table. The factor χ^2 is calculated for the reading to be tested and if it exceeds 3·841 then the difference in distribution in the two tubes is taken as significant. Again only one reading or the mean of several readings may be tested in this manner.

If, as in most of the experiments, the difference between the numbers swimming up in the pair of tubes is too small to satisfy this test for significance, it is possible to lump together the single readings from each one of a series of comparable experiments and perform the test on these larger figures. Reliance can only be placed on this 'pooled result' if the data are shown independently to satisfy a test for homogeneity.

EXPERIMENTAL RESULTS

The Horizontal Apparatus

The results of three typical experiments in the straight horizontal apparatus are given in Tables II–IV. The first comprises the first day's readings in a control experiment having both tubes uniformly filled with water freshly taken from the Plymouth Laboratory circulation ('tank water') and with ten *Hemimysis lamornae* introduced into each side of both tubes at 12.00 hr. It is seen that the distribution remains fairly uniform, and although two readings (12.30 and 13.00 hr. in tube II) show a significant departure from normality this is not maintained. Such significant clumpings in a uniform tube are referred to later.

Table III gives the readings in an experiment with water rich in the flagellate *Rhodomonas* sp. in the left-hand side of one tube and ultrafiltered water in the right-hand side; and *Rhodomonas* in the right-hand side of the other and ultrafiltered water in the left. Ten *Hemimysis lamornae*, previously fed on diatoms and flagellates, were introduced into each side of both tubes at 10.15 hr. An immediate movement away from the flagellates occurred and this was maintained for the duration of the experiment.

TABLE II. EXPERIMENT DHT 30 (11 March 1952)

(Two horizontal tubes set up as described. Both completely filled with fresh tank water. Ten *Hemimysis lamornae* put in each side of both. Just taken from the aquarium tanks.)

Time (hr.)	I				II			
	A	B	C	D	A	B	C	D
	Normal		Normal		Normal		Normal	
12.00	—	10	10	—	—	10	10	—
12.30	8	1	1	10	4	1	2	13
13.00	7	1	1	11	5	—	4	11
14.30	8	2	2	8	8	2	3	7
16.00	8	1	3	8	12	—	1	7
17.30	8	2	3	7	11	4	1	5
19.00	8	3	2	7	10	4	2	4
20.30	—	12	7	1	1	11	7	1

TABLE III. EXPERIMENT DHT 31 (13 March 1952)

(Two horizontal tubes set up as described. I, *Rhodomonas* sp. culture in L.H.S. Ultrafiltered water in R.H.S. II, ultrafiltered water in L.H.S. *Rhodomonas* in R.H.S. Ten *Hemimysis lamornae* put in each side of both. Previously fed on a mixture of diatoms and flagellates.)

Time (hr.)	I				II			
	A	B	C	D	A	B	C	D
	Rhodomonas		Normal		Normal		*Rhodomonas*	
10.15	—	10	10	—	—	10	10	—
10.30	1	—	7	12	10	7	3	—
11.00	1	—	7	12	14	4	2	—
11.30	1	—	5	14	15	3	2	—
13.00	1	—	7	12	13	5	1	1
14.30	1	1	7	11	12	6	2	—

TABLE IV. EXPERIMENT DHT 32 (14 March 1952)

(Two horizontal tubes set up as described. I, *Thalassiosira gravida* culture in L.H.S. Ultrafiltered water in R.H.S. II, ultrafiltered water in L.H.S. *Thalassiosira* culture in R.H.S. Ten *Praunus neglectus* put in each side of both. Previously fed on a mixture of diatoms and flagellates.)

Time (hr.)	I				II			
	A	B	C	D	A	B	C	D
	Diatom		Normal		Normal		Diatom	
11.00	—	10	10	—	—	10	10	—
11.30	9	5	1	5	7	2	2	9
12.00	11	5	1	3	5	2	3	10
13.00	10	6	1	3	3	1	7	9
14.30	11	7	—	2	—	—	10	10
16.00	13	5	1	1	—	—	8	11

Table IV gives the readings in an experiment with water enriched with the diatom *Thalassiosira gravida* in one side of each of the two tubes, and filtered water in the other, and with ten *Praunus neglectus* put in each side. A marked migration is again apparent, but in this case into the enriched water and rather slower in occurrence. All but the first reading in tube I and the first two in tube II are significant.

TABLE V. EXPERIMENT HCT 4 (16 October 1951)

(Horizontal circular tube set up as described. Completely filled with fresh tank water. Twelve *Hemimysis lamornae* put in at each junction. Previously fed on *Peridinium trochoidium*.)

Time (hr.)		A	B		C	D		E	F	
		Normal			Normal			Normal		
12.30	6			12			12			6
12.45		4	4		7	8		8	5	
13.00		6	2		8	7		8	5	
14.15		3	2		7	7		11	6	
16.00		3	2		11	7		8	5	
16.30		2	3		12	6		8	5	
17.00		3	5		8	6		9	5	
17.45		4	3		11	5		7	6	
18.30		1	5		10	4		11	5	
19.00		2	6		8	6		8	6	
20.45		3	4		3	7		11	8	
21.30		7	4		5	6		6	8	

TABLE VI. EXPERIMENT HCT 3 (15 October 1951)

(Horizontal circular tube set up as described. Water rich in *Peridinium trochoidium* in one third. Outside water in the rest. Twelve *Hemimysis* put in at each junction. Starved in filtered water for the previous 4½ hr.)

Time (hr.)		A	B		C	D		E	F	
		Normal			Flagellate			Normal		
15.00	6			12			12			6
15.15		2	3		10	12		5	4	
15.30		3	3		11	11		4	4	
16.00		3	4		7	13		7	2	
16.30		1	2		9	14		7	3	
17.00		1	5		4	19		6	1	
17.30		1	4		5	17		5	4	
18.00		—	4		8	14		6	4	
18.30		2	3		7	16		4	4	
19.00		4	3		9	12		6	2	
22.45		4	3		5	10		7	7	

For comparison with these three experiments, the results of two performed in the horizontal circular tube are given in Tables V and VI. The first is a control experiment with the tube completely filled with tank water and twelve *Hemimysis lamornae* put in at the three entrances; and the second has one-third filled with water enriched with *Peridinium trochoidium* culture and the *Hemimysis* used had previously been starved in ultrafiltered water. Distribution in the control experiment in relation to sector CD departs significantly from normality in only one reading, that of 16.30 hr. In the other, however, a clear movement into the enriched water sector CD takes place at once and is maintained for the greater part of the experiment.

These, and the remaining horizontal results, have been treated mathematically and the experiments put into one of the three categories—*positive*, *negative*, or *not significant*—depending upon whether a significant migration into, or away from, the phytoplankton takes place, or whether there is no significant departure from an even distribution. The experiments are classified first according to the animals used, and secondly, according to the phytoplankton organisms or the type of water. They are recorded in the following tables, together with details of the concentration of phytoplankton put in the one side of the apparatus and the state of the animal. This latter is taken as either starved or fed dependent upon whether the animals had been kept for some time in filtered water, or had been fed or just recently caught (when food is generally to be found in the gut).

The tables may best be considered according to the animals used and *Hemimysis lamornae*, with which the greatest number of experiments was performed, is taken first.

Hemimysis lamornae

Experiments involving Diatoms

Amongst the diatoms, clear migrations into water enriched with *Skeletonema costatum*, *Thalassiosira gravida*, *Biddulphia sinensis*, *Nitzschia closterium* and a mixed culture of diatoms were obtained. There were no negative migrations amongst these species but out of nineteen experiments performed five gave no significant result. The distribution of the positive results between fed and starved animals suggests a more marked migration by the latter, but the results do not satisfy a mathematical test for significance. Exp. DHT 51 has been disregarded, as later work showed animals to move away from ordinary water passed through the Berkefeld filter candle employed here. This is considered later (p. 407). Five experiments were performed with *Lauderia borealis*, of which three proved not significant, one positive and one just negative (four significant negative readings out of a total of eight). *Lauderia* was not used in any other experiments, but it may be that it is not particularly acceptable to *Hemimysis*. There was no special indication of a lethal effect in the 'toxicity experiments'. *Coscinodiscus concinnus* also gave two not significant results, but more experiments would need to be performed before it could be said not to be acceptable to the animals. Excluding these two doubtful species, the summarized results with diatoms reveal a substantial majority of experiments showing a positive migration. Even including them there is only one negative migration as compared with fifteen positive in a total of twenty-six.

Two of the experiments with *Skeletonema* and two with *Thalassiosira* utilized water from a growing culture which was centrifuged in order to remove the bulk of the cells. Positive migrations were still obtained in three of these four cases suggesting that the attractive principle may reside in the water itself.

TABLE VII. *HEMIMYSIS LAMORNAE* AND DIATOMS

(In this and the subsequent tables the four columns represent: (i) the serial number of the experiment; (ii) the concentration, in cells/mm.^{3}, of the phytoplankton used; (iii) the state of the animal, F representing fed and S starved; (iv) the result of the experiment as a whole. If there should be insufficient significant readings to merit designating the whole experiment such, or if only a minimum number should be so, then this may be noted as e.g. (3 rds +ve) meaning three significant positive readings.) N.S.: not significant.

Skeletonema costatum

DHT 22	10	F	+ve	DHT 68	Centrifuged	F	+ve
...	10	F	N.S.	...	Centrifuged	F	+ve
DHT 23	10	S	+ve	[DHT 51	Filtered	F	−ve]
...	10	S	+ve	...	Filtered	F	−ve

Thalassiosira gravida

DHT 24	147	F	N.S.	HCT 18	55	S	+ve
...	147	F	N.S.	DHT 70	Centrifuged	F	N.S.
HCT 16	90	S	+ve	...	Centrifuged	F	+ve

Lauderia borealis

DHT 52	21	S	N.S.	DHT 55	2¼	S	N.S.
...	21	S	−ve*	...	2¼	S	N.S.
HCT 1	20/ml.	F	+ve				

* 4 rds.

Biddulphia sinensis

P 63	275	F	+ve	P 61	220	S	+ve
P 62	220	F	N.S.*	P 60	220	S	+ve

* In dark.

Coscinodiscus concinnus

2P 5	28/ml.	F	N.S.	2P 6	28/ml.	S	N.S.

Mixed diatoms

HCT 48	700	F	+ve	HCT 49	350	F	+ve *

* Halves.

Nitzschia closterium

P 64	3500	S	+ve

Summary distribution of results with *H. lamornae* and diatoms

	All species				Excluding *Lauderia* and *Coscinodiscus*		
	+ve	N.S.	−ve		+ve	N.S.	−ve
Fed	8	6	—	Fed	7	5	—
Starved	7	4	1	Starved	7	—	—

Experiments involving Flagellates

The independent evidence of a possible toxic action by some of the flagellates used is taken as the basis for a division of the remaining results involving phytoplankton and *Hemimysis* into two groups—those involving 'toxic flagellates' and those involving 'non-toxic flagellates'. The latter will be considered first.

As seen in Table VIII, significant migrations were obtained into concentrations of *Chlamydomonas* sp., *Peridinium trochoidium*, *Pyramimonas* sp., *Dicrateria inornata* and Flagellate K. Again a greater proportion of positive results was obtained with starved than with fed *Hemimysis*, but not significantly so. There was a greater number of not significant results even with the starved animals, and the totals are fifteen positive results to twenty-one not significant. Four experiments with *Syracosphaera carterae* and two with *Exuviaella baltica* all gave not significant results. A 'toxicity experiment' was not performed with *Syracosphaera* but there was no indication of a harmful

TABLE VIII. *HEMIMYSIS LAMORNAE* AND 'NON-TOXIC FLAGELLATES'

Chlamydomonas sp.

2P 11	520	F	N.S.	DHT 46	42	F	N.S.
HCT 25	225	F	N.S.	...	42	F	N.S.
P 30	170	F	N.S.	DHT 48	30	F	N.S.
DHT 49	113	F	N.S.	...	30	F	N.S.
...	113	F	N.S.	2P 12	520	S	+ve
DHT 45	105	F	+ve	HCT 24	250	S	+ve
...	105	F	+ve	HCT 21	235	S	+ve
DHT 50	77	F	N.S.	P 29	170	S	+ve
...	77	F	N.S.	HCT 72	120	S	N.S.
DHT 47	62	F	N.S.	DHT 53	60	S	N.S.
...	62	F	N.S.		60	S	N.S.

Peridinium trochoidium

DHT 1	53*	F	+ve	HCT 3	18	S	+ve
DHT 2	28*	F	+ve	HCT 5	18	S	N.S.
...	28*	S	N.S.				

* Mixed with *P. micans* and *P. triestinum*.

Pyramimonas sp.

DHT 41	139	F	+ve	DHT 42	139	S	N.S.
...	139	F	+ve	...	139	S	N.S.

Syracosphaera carterae

HCT 51	68	F	N.S.	HCT 19	77	S	N.S.
HCT 70	8	F	N.S.	HCT 20	70	S	N.S.

Exuviaella baltica

HCT 40	2	F	N.S.	DHT 21	31	S	N.S.

Dicrateria inornata

2P 4	2760	F	+ve	2P 3	2040	S	+ve
2P 2	3130	S	+ve				

Flagellate K

HCT 57	230	F	+ve*	HCT 71	10	S	N.S.†

* 4 rds.

† 3 rds −ve.

Summary distribution of results with *H. lamornae* and non-toxic flagellates

All species

	+ve	N.S.	−ve
Fed	8	16	—
Starved	7	11	—

Excluding *Syracosphaera* and *Exuviaella*

	+ve	N.S.	−ve
Fed	8	13	—
Starved	7	8	—

effect in the horizontal tubes; one performed with *Exuviaella* revealed no effect. It may be that these two forms fall into the same 'unacceptable' category as

TABLE IX. *HEMIMYSIS LAMORNAE* AND 'TOXIC FLAGELLATES'

Gymnodinium II

HCT 58	54	F	N.S.	DHT 69	Centrifuged	F	−ve
HCT 50	40	F	N.S.*	...	Centrifuged	F	−ve
HCT 47	14	F	+ve	DHT 71	Cent. (dil.)	F	−ve
HCT 46	7	F	N.S.	...	Cent. (dil.)	F	N.S.
[DHT 37	Filtered	F	−ve	DHT 35	63	S	N.S.‡
...	Filtered	F	N.S.†]	...	63	S	N.S.
				2P 20	—	S	N.S.

* *Gymno.* I. † 2 rds −ve. ‡ 3 rds −ve.

Flagellate 12

DHT 18	98	F	N.S.*	[DHT 36	Filtered	F	−ve
...	98	F	N.S.	...	Filtered	F	N.S.]
HCT 53	37	F	−ve	DHT 19	98	S	N.S.
HCT 52	18	F	N.S.	...	98	S	+ve
HCT 37	6	F	N.S.	DHT 20	98	S	N.S.
DHT 64	Centrifuged	F	N.S.	HCT 66	29	S	N.S.
...	Centrifuged	F	+ve				

* 3 rds −ve.

Oxyrrhis marina, *Rhodomonas* sp. and *Nitzschia closterium*

HCT 31	20·5	F	+ve	DHT 20	80	S	N.S.
HCT 56	16·0	F	N.S.	DHT 25	60	S	−ve
HCT 35	15·0	F	N.S.	...	60	S	−ve
HCT 33	12·0	F	N.S.	2P 10	*c.* 4·0	S	+ve
P 33	11·0	F	N.S.	DHT 26	40	F	−ve
P 69	7·0	F	N.S.*	...	40	F	−ve
HCT 55	6·5	F	N.S.	DHT 27	23	F	−ve
HCT 54	5·5	F	N.S.	DHT 28	Aerated	F	N.S.†
2P 9	*c.* 4·0	F	N.S.	DHT 29	65	F	+ve‡

* +ve tend. † −ve finally. ‡ No *Rhodomonas*.

Rhodomonas only (*Oxyrrhis* filtered off)

DHT 29	200	F	−ve*	DHT 56	1	S	−ve
DHT 31	100	F	−ve	...	1	S	−ve
...	100	F	−ve	[DHT 27	Filtered	F	−ve]

* 4 rds.

Chlorella stigmatophora

DHT 33	3580	F	+ve	DHT 34	3800	S	+ve
...	3580	F	N.S.	...	3800	S	N.S.

Summary distribution of results with *H. lamornae* and toxic flagellates (excluding *Chlorella*)

	+ve	N.S.	−ve
Fed	3	17	12
Starved	2	7	4

Lauderia and *Coscinodiscus*. In none of this group of experiments was any negative migration obtained and, although there are more not significant results than positive, the positive bias is still most marked.

The 'toxic flagellate' group comprises the two cultures of *Gymnodinium* (I and II) and Flagellate 12, all showing lethal effects in the toxic experiments; and the mixed culture of *Oxyrrhis marina*, *Rhodomonas* sp. and *Nitzschia closterium* which, although not showing a toxic effect in the weak culture used in the test experiments, afterwards showed enough harmful effects in the horizontal tubes to merit its inclusion here. The position of *Chlorella* is anomalous both on account of its being a non-motile chrysophycaean and because the experiments performed with it were at the optimum concentration which showed little toxic effect. The results with it are therefore considered separately.

All but one of the *Gymnodinium* experiments involved *Gymnodinium* II, the species showing the more marked toxic effect. Out of eleven experiments one gave a positive result (with fed animals) and three gave negative results. The remainder were all not significant. The positive result was obtained with a low concentration of cells and the negative results with water from a growing culture which had been centrifuged to remove most of the cells. The centrifugate used in DHT 71, where only one of a pair was significantly negative, was previously diluted with ordinary water. The inference may be made that the repellent principle is situated in the water in which the flagellate has grown.

Of eleven results with Flagellate 12 the majority again are not significant. One is negative, involving fed animals; and two positive, one normal with starved animals and one with centrifuged water and fed animals. The latter would seem opposed to the idea of a toxic or repellent principle in the water, and in fact the form of the gut in most specimens (which fed freely) suggested some internal effect, but the evidence is rather tenuous. Unfortunately, the only cultures of Flagellate 12, which were at Plymouth, Millport and Bangor, died out at about the same time in the spring of 1952. The results will, however, be followed up if it proves possible to re-establish a culture from the sea. This organism is of particular interest as it is thought to be a primitive dinoflagellate, a group which embraces the gymnodiniums, *Oxyrrhis* and other forms such as *Goniaulax*, which are known to have toxic properties.

Three positive results were obtained with the *Oxyrrhis*, *Rhodomonas* and *Nitzschia* culture, but ten negative ones: all out of a total of twenty-three. *Oxyrrhis* is not an autotrophic flagellate and can only be grown if supplied with food. This particular culture provides a large *Nitzschia* and the autotrophic *Rhodomonas* as possible food and is of long standing. The *Nitzschia* content fluctuates a great deal but is generally low, while most *Rhodomonas* cells clump into aggregations about the size of a small tea leaf, and the *Oxyrrhis* swims freely. The culture was used just as grown in the early experiments when both positive and negative results were obtained. These led to an attempt to separate the component parts in order to assess their individual effects, and this was found to be relatively easily done by filtering through fine silk. A growth was obtained with a low *Nitzschia* content, and after being left quiescent for some days to ensure a maximum clumping of the *Rhodomonas*, was passed through fine silk. Almost pure *Oxyrrhis* passed through and the *Rhodomonas* clumps were left on the silk. They were washed with culture solution and then resuspended and shaken vigorously in filtered sea water. This treatment gave a suspension containing a good many free cells. Consistent and very marked migrations away from such a suspension were obtained, while the almost pure *Oxyrrhis* gave a positive result. It seems clear that the variable results with the mixed culture derived from the opposing reactions to the *Oxyrrhis* and the *Nitzschia* on the one hand and to the *Rhodomonas* on the other. The two positive migrations with the mixed culture were both into samples with a low *Rhodomonas* content as compared with the *Oxyrrhis*.

The summary of results with the toxic flagellates shows the preponderance of negative migrations as compared with positive. These result largely from the effect of *Rhodomonas* and *Gymnodinium* II, both of which may be considered to be avoided because of their harmful properties.

Four experiments were performed with *Chlorella stigmatophora* at its optimum concentration for survival, two with fed *Hemimysis* and two with starved. One of each of these pairs showed a significant positive migration, the other being not significant.

Miscellaneous Experiments

An earlier experiment (HCT 9) used small zooplankton animals in place of phytoplankton. Some of these died and a concentration of bacteria quickly formed in the tube and was apparently avoided by the *Praunus neglectus* being tested. This led to a closer study of the effect of water rich in bacteria.

TABLE X. *HEMIMYSIS LAMORNAE* AND BACTERIA

DHT 3	Normal bacteria	F	−ve
...	Normal bacteria	F	−ve
DHT 9	Normal bacteria	—	−ve
DHT 14	Normal bacteria	—	N.S.*
DHT 7	Diluted to 50 %	—	−ve
...	Diluted to 50 %	—	−ve
DHT 65	Diluted to 25 %	F	−ve
...	Diluted to 25 %	F	−ve
DHT 6	Diluted to 10 %	F	N.S.
...	Diluted to 10 %	—	N.S.
DHT 8	Filtered	—	−ve
...	Filtered	—	−ve
DHT 4	Aerated (60 min.)	—	N.S.
...	Aerated (60 min.)	—	N.S.
DHT 9	Aerated (5 min.)	—	−ve
DHT 14	Aerated (45 min.)	—	−ve
DHT 16	Aerated (45 min.)	—	N.S.†
DHT 67	N_2 bubbled	F	N.S.
...	N_2 bubbled	F	N.S.
DHT 17	Bubbled over from bacteria	—	N.S.

* All dead in bacteria.

† Using new diffuser.

A suspension of bacteria was grown by leaving some small dead animal, such as a prawn, in a breffit of raw sea water for about 24 hr. This gave rise to a slightly cloudy water with a distinctive but not overpowering smell. No attempt was made to identify the species producing this putrefaction, but on separate occasions the method of growth gave a culture consistent in appearance and presumably in composition, although it appeared at times more potent than at others.

As is shown in Table X this type of culture produced a marked and sustained avoiding reaction in the *Hemimysis* used. On occasions, as in DHT 14, when the culture was especially strong, deaths might even be caused. The negative migration persisted when the culture was diluted first to 50% strength and then to 25%; but when diluted to 10% no significant result was obtained. In an attempt to define more precisely the agent inducing this reaction a culture was passed through an ultra-filter, when a negative migration was still obtained. If a culture was aerated for 60 min. before use, however, no significant result was obtained, although aeration for a shorter time did not seem adequate to remove the noxious agent, until a much more efficient diffuser and method of bubbling were adopted. The destruction or removal of the material inducing the avoidance by bubbling with air, left the possibility of its being oxidized or perhaps carried away in the stream of escaping bubbles. A culture was therefore bubbled with nitrogen and then tested in the apparatus (DHT 67), when no significant result was obtained. It may thus be concluded that the substance avoided is a volatile, water-soluble metabolic or decomposition product of the bacteria. In the light of this conclusion an attempt was made to remove the substance from a culture by bubbling and to reabsorb it in filtered water which could then be used in the apparatus. No significant movement in relation to such water was demonstrated.

With the above evidence that some of the migrations in relation to bacteria and phytoplankton were perhaps mediated by substances dissolved in the water, attempts were made to determine the reactions of *Hemimysis* to one or two simple substances.

The first of these tested was carbon dioxide. Sea water was saturated with carbon dioxide by bubbling with gas from a cylinder (and washed before use). When such saturated water was put into the apparatus and animals introduced, those going into the carbon-dioxide-rich side went immediately into convulsions and died within a matter of seconds without being able to escape to the other side of the apparatus. Those animals put into the other side made no attempt to move into the carbon-dioxide-rich water. When the saturated water was diluted down to 10% with normal water and the animals introduced, then a rapid and sustained negative migration took place without any deaths. An equally clear reaction was got with a 5% dilution, but not with 1 and ½%, when no significant movement resulted. The pH of the ½% dilution was about 8·0, which is similar to that of the bacteria-rich water. The pH of the 5% dilution was about 7·2.

The second series tested migration in relation to degree of aeration. One sample of water was supersaturated by shaking vigorously, and another deaerated by subjecting to the reduced pressure of a powerful filter-pump together with shaking. When these two lots of treated water were put into opposite sides of the apparatus and animals introduced, then no significant departure from a uniform distribution was obtained. Similar results obtained with water deaerated by this means in one side and normal water in the other. If the water was deoxygenated by bubbling with washed nitrogen from a cylinder for 45 min., then animals going into that side of the apparatus died within minutes but made no attempt to escape to the normal side. There were in this case no convulsions, and death was much slower than with carbon dioxide. All movement ceased almost at once and the animals lay on their sides and gradually became opaque. If they were removed within about 5 min. and put into well-aerated water, then a good proportion recovered completely. The nitrogen bubbled water was diluted down to 28%; and water bubbled for 20 min. was diluted down to 25% with normal water but no avoiding reaction towards either could be demonstrated, while no further deaths took place at these concentrations.

Table XI. *Hemimysis lamornae* and Miscellaneous Substances

			Carbon-dioxide-rich water				
DHT 5	10% saturated	—	−ve	DHT 13	1% saturated	—	N.S.
DHT 11	5% saturated	—	−ve	DHT 12	½% saturated	—	N.S.
...	5% saturated	—	−ve	...	½% saturated	—	N.S.
DHT 13	1% saturated	—	N.S.				
			Aerated and deaerated water				
DHT 10	Aerated* and deaerated†	—	N.S.	DHT 61	28% N_2 bubbled	—	N.S.
...	Aerated* and deaerated†	—	N.S.	...	28% N_2 bubbled	—	N.S.
DHT 15	Normal and deaerated†	—	N.S.	DHT 66	25% of 20 min. N_2 bubbled	—	N.S.
...	Normal and deaerated†	—	N.S.	...	25% of 20 min. N_2 bubbled	—	N.S.
DHT 60	N_2 bubbled 45 mins.	—	N.S.‡				
...	N_2 bubbled 45 mins.	—	N.S.‡				
			Ammonia-rich water				
DHT 62	6 drops of 10% NH_3/litre	F	+ve	DHT 63	12 drops of 10% NH_3/litre	F	+ve
...	6 drops of 10% NH_3/litre	F	+ve	...	12 drops of 10% NH_3/litre	F	+ve
			Filter etc.				
DHT 54	Mysid inhabited water	F	N.S.	DHT 58	Berkefeld filtered water	F	−ve
...	Mysid inhabited water	F	N.S.	...	Berkefeld filtered water	F	−ve
DHT 57	Berkefeld filtered water	F	N.S.	DHT 59	Membrane filtered water	S	N.S.
...	Berkefeld filtered water	F	−ve	...	Membrane filtered water	S	N.S.

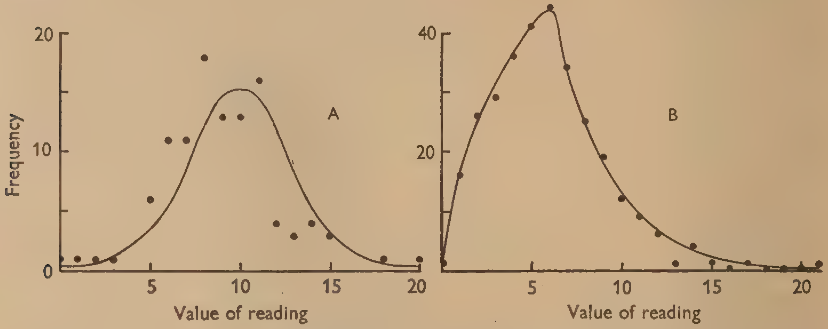
* Shaking. † Reduced pressure. ‡ Animals died at once.

The possibility of ammonia being one of the bacterial decomposition products led to its being tested in the apparatus. 0·880 ammonia was diluted to 10% with sea water and six drops of this solution were added per litre of sea water, giving a pH of just over 8·8. This water was placed in one side of the apparatus and normal water in the other. The *Hemimysis* introduced moved immediately into the ammonia concentration and remained there. A precisely similar result was obtained using double the concentration of ammonia (12 drops per litre).

Miscellaneous tests included one on water in which mysids had lived for some time. No significant migration in relation to this was obtained. The experiments referred to earlier as involving filtered water from various cultures, used water passed through a coarse Berkefeld plaster filter candle. The water was drawn through by suction with a siphon about 5 ft. long. The high proportion of negative results obtained with such water led to the filter itself being tested in the following manner. Previously ultra-filtered water (from the laboratory supply) was passed through the filter in the usual manner and put in one side of the apparatus, while unrefiltered water was put in the other. A marked negative migration in relation to such water was obtained on three occasions out of four. As a result of this test it was decided to disregard the experiments using water filtered in this way except for the first one involving bacteria water. It was advised that such plaster filters are liable to absorb substances from the water passed through them and later give them up to other water. It seems probable that something was absorbed from the bacteria culture and released afterwards to contaminate the various types of water passed through it. The pores of the filter would also hold organic matter of various kinds from the diatoms, etc., and this could decompose and then contaminate other water. Water filtered through a membrane filter was tested and found to induce no significant migration. It did not prove practicable, however, to use this method for filtering cultures, as it was extremely slow. It was finally decided to use the centrifuging method already referred to in order to remove cells and leave the culture otherwise unaffected.

Besides the above experiments involving cultures and chemical substances, fourteen control experiments were performed with the apparatus uniformly filled with water of some kind. None of these resulted in a whole experiment being termed significant but occasionally, as already mentioned in DHT 30 and in HCT 4, a temporary distribution differing significantly from a uniform one was obtained. The frequency with which each particular reading occurred in the nine experiments done in the straight horizontal apparatus is shown in Text-fig. 2A. The form of the curve drawn through the points is typically that which would be expected were the distribution of the animals in the tube random. Such significant departures from a uniform distribution as do occur in the control experiments could perhaps be a manifestation of the shoaling habit which is common in these animals, but this tendency is not marked enough to appear in the figure. The second curve (Text-fig. 2B) shows the frequency with which each particular reading occurred in three control experiments done in the circular horizontal tube. This includes all the readings for each of the six sectors, and is asymmetrical because the one or two high readings (to be expected by chance) each necessarily result in there being several low readings, since the remaining few animals have to be distributed amongst the other five sectors.

It may therefore be concluded from the control experiments that in the uniform tubes there is no tendency to aggregate in any particular section, and those significant departures from an even distribution which are found in the remainder of the experiments are due to the environmental conditions in the tubes.



Text-fig. 2. Summary of control experiments with *Hemimysis lamornae*. A, frequency of occurrence of readings in Exps. DHT 1, 30, 38, 39 and 40. B, the same in Exps. HCT 2, 4 and 17.

Other mysids

Experiments were performed with four other species of mysid—*Praunus neglectus*, *P. flexuosus*, *Neomysis integer* and *Mesopodopsis slabberi*. The behaviour of these species appearing to be similar, and rather few experiments having been done with each, it seems most convenient to consider all the results together.

Experiments involving Diatoms

As seen from the summary distribution of results (Table XII), out of twenty-four experiments involving various diatoms twenty gave significant positive migrations and four gave no significant result. Movement was most marked into *Nitzschia closterium* (the minute Plymouth strain) and *Thalassiosira gravida*; while neither of two experiments performed with *Eucampia zoodiacus* gave a significant result. One of the experiments with *Neomysis integer* was performed in the dark and gave no significant migration until the apparatus was moved into the light. The distribution then became positive. In one of the experiments with *Neomysis* the diatom culture was deoxygenated before use by subjecting it to reduced pressure combined with shaking and in another the diatoms were killed by heating to 80° C., washed on a filter and

TABLE XII. OTHER MYSIDS AND PHYTOPLANKTON, ETC.

(1) *Praunus neglectus*

Nitzschia closterium			
HCT 60	3000	F	+ve
HCT 65	440	F	+ve
HCT 63	2300	S	+ve*
Thalassiosira gravida			
DHT 32	115	F	+ve
..	115	F	+ve
Chlorella stigmatophora			
HCT 43	1210	F	N.S.
HCT 61	1840	S	+ve†
Eucampia zoodiacus			
HCT 69	1½	F	N.S.
Oxyrrhis marina, Rhodomonas sp. and *Nitzschia closterium*			
HCT 32	11	F	+ve
HCT 34	7	F	N.S.
HCT 62	34	S	+ve
HCT 64	17	S	+ve
Chlamydomonas sp.			
HCT 27	275	S	+ve
HCT 68	200	S	+ve*

Flagellate 12			
HCT 38	8	F	N.S.
HCT 41	11	S	+ve†
Flagellate L4 (13/4) 2L Clone 6			
HCT 44	650	F	N.S.
HCT 45	505	S	+ve
Exuviaella baltica			
HCT 42	5	F	N.S.
DHT 21	31	S	+ve
Zooplankton			
HCT 36	—	F	N.S.
HCT 28	—	S	N.S.
HCT 39	—	S	N.S.
Bacteria			
HCT 59	—	F	−ve†
Ammonia			
HCT 73	—	S	N.S.

* Halves. † 4 rds.

(2) *Praunus flexuosus*

Eucampia zoodiacus			
HCT 11	1·4	S	N.S.
Chlamydomonas sp.			
P 66	810	F	N.S.
Zooplankton			
HCT 9	—	S	−ve

Thalassiosira gravida			
HCT 13	120	S	+ve
HCT 14	100	S	N.S.
Bacteria			
HCT 12	—	F	−ve

(3) *Neomysis integer*

Nitzschia closterium			
H 9	1090	F	+ve
H 19*	1080	F	N.S.
H 25	1060	F	+ve
H 20	1060	(F)	+ve
H 27	1060†	F	+ve
H 23	1020	F	+ve
H 17	800‡	(F)	+ve
H 4	590	(F)	+ve
H 13	1600	(S)	+ve
H 10	1090	S	+ve
H 26	1060	S	+ve
H 22	1020	S	N.S.§
DHT 18*	800	S	+ve
DHT 28	Killed	S	+ve
DHT 12	400 (and alga)	(S)	+ve

Non-motile green alga			
H 14	1400	S	+ve
H 6	50	(F)	N.S.
Miscellaneous			
H 24	1060 *Nitzschia* and tinted	F	N.S.
H 5	830 *Nitzschia* and tinted	(F)	N.S.
H 7	830 *Nitzschia* and tinted	S	+ve
H 8	Filtered *Nitzschia*	(S)	N.S.
H 21	Filtered *Nitzschia*	(F)	N.S.
H 29	Kieselguhr	(S)	N.S.
H 11	Tinted water	(S)	N.S.

N.B. When the letters F and S are bracketed in this table the probable state of the animals is indicated.

* Half *N. integer* and half *Mesopodopsis slabberi*.
‡ Cells resuspended.
† Culture deoxygenated.
§ +ve in light (performed largely in dark).

Table XII (*continued*)

Summary distribution of results with other mysids.

Diatoms

	+ve	N.S.	−ve
Fed	11	2	—
Starved	9	2	—

Non-toxic flagellates

	+ve	N.S.	−ve
Fed	—	5	—
Starved	6	—	—

Toxic flagellates

	+ve	N.S.	−ve
Fed	1	2	—
Starved	3	—	—

resuspended in filtered water before use. In both these experiments marked positive migrations were still obtained. This whole series is distinctive for the high proportion of positive results, there being no negative ones and no apparent difference in behaviour between fed and starved animals.

Experiments involving Flagellates

Of eleven experiments with typically 'non-toxic' flagellates five results, all with fed animals, were not significant and six, all with starved animals, were significantly positive. These include two experiments with *Chlorella stigmatophora* rather below its optimum concentration, and two with an unidentified non-motile green alga which was probably a *Chlorella*. Of two experiments with *Exuviaella baltica*, which had induced no positive migration in *Hemimysis lamornae*, one significant positive result was obtained, this being with starved animals and a much higher concentration of cells than used previously. The other positive results were with *Chlamydomonas* and the flagellate L 4 (13/4) 2L Clone 6. Again there were no negative results.

Finally, of four experiments with the mixed *Oxyrrhis* culture and two with Flagellate 12, both in the 'toxic' group, four positive results were obtained, the two not significant ones being with fed animals. The Flagellate 12 concentrations used were lower than those used previously, and the proportions of *Rhodomonas* to *Oxyrrhis* were generally low so this perhaps may account for the absence of negative migrations amongst these experiments.

Miscellaneous Experiments

Amongst the various miscellaneous experiments reported in Table XII is the one involving *Praunus flexuosus* and a concentration of zooplankton which led to the series using bacteria. In this there was no evidence of any movement into the sector rich in zooplankton taken from a townet haul nor was there in three other similar experiments with *P. neglectus*, all of which gave not significant results. The negative movement in the *P. flexuosus* experiment must almost certainly be taken as being induced by the growth of bacteria which took place in the tube. This growth, although making the water slightly cloudy, was not sufficient to kill any of the animals used. Two experiments with independently produced bacteria cultures confirmed the negative migration.

An experiment with the non-motile alga culture in one side and *Nitzschia* in the other showed a clear migration into the latter, despite the not unattractive nature of the alga as shown in an independent experiment. One involving a suspension of Kieselguhr (diatomaceous earth) and also six control experiments using various animals all gave no significant results, as did three with water tinted with water colours to resemble a diatom culture.

TABLE XIII. *ARTEMIA SALINA* AND DIATOMS AND FLAGELLATES

Nitzschia closterium

P 42	3720	F	N.S.[*]
2P 15	2380	F	N.S.
2P 14	2340	F	N.S.[†]
2P 16	1800	F	N.S.[‡]
DHT 44	480	F	N.S.[§]
...	480	F	+ve
DHT 43	43	F	N.S.
...	43	F	+ve
2P 17	1005	S	N.S.
P 43	820	S	N.S.[‖]

Hemiselmis rufescens

2P 19	3500	S	N.S.
2P 18	2820	S	+ve

Flagellate 4

HCT 7	72	S	N.S.
HCT 8	70	S	N.S.

[*] −ve 2nd day.
[†] Poor gradient.
[‡] −ve 2nd day.
[§] 2 rds +ve.
[‖] 3 rds +ve.

Summary distribution of results with *Artemia salina* and diatoms and flagellates.

	+ve	N.S.	−ve
Fed	2	6	—
Starved	1	5	—

Artemia salina

Fifteen experiments were performed using specimens of *Artemia salina*, the brine shrimp, which had been reared in captivity at Plymouth. Of ten of these involving *Nitzschia closterium*, two gave significant positive results (both with fed animals) and two had positive tendencies; the remainder, with the exception of two with negative tendencies on the second day, being not significant (Table XIII). Of two experiments with *Hemiselmis rufescens*, one was significantly positive and of two with Flagellate 4 both were not significant. Most of the results were thus not significant but again there were no negative results.

Decapod larvae

Twelve experiments with decapod larvae gave much more variable results. Of nine with cultures not considered toxic two positive and two negative results were obtained; and of three with the mixed *Oxyrrhis* culture, one positive, one negative and one not significant. These experiments were done with various kinds of zoea and megalopa larvae, but there did not appear to be any correlation between the species used and the type of result obtained. There may perhaps not be enough experiments in the series for the usual trends to become apparent, but it seems more likely that a rather different type of behaviour is being encountered.

TABLE XIV. DECAPOD LARVAE AND DIATOMS AND FLAGELLATES

Nitzschia closterium				
P 77	4400	S	N.S.	*Leander* larvae
P 26	2720	S	N.S.	Zoeas
Thalassiosira gravida				
P 75	10	S	−ve	Zoeas
Dicrateria inornata				
2P 7	3440	F	N.S.	*Carcinus* zoeas
2P 1	3130	F	+ve	*Carcinus* zoeas
P 74	4620	S	N.S.	Zoeas
P 16	2250	S	−ve	—
P 27	1280	S	+ve	Zoeas and megalopas
P 25	1220	S	N.S.	Zoeas
Oxyrrhis marina, *Rhodomonas* sp. and *Nitzschia closterium*				
P 76	36	S	−ve	—
P 79	35	S	+ve	*Porcellana* zoeas
P 80	35	S	N.S.	*Porcellana* zoeas

Summary distribution of results with *Decapod larvae* and all diatoms and flagellates

	+ve	N.S.	−ve
Fed	1	1	—
Starved	2	5	3

Small copepods, etc.

The six experiments performed with small copepods of various kinds all gave significant positive migrations, one being with fed animals (Table XV). The three diatoms used in this series have all on other occasions seemed acceptable to animals, and the high incidence of positive results may perhaps depend upon this and the fact that many more animals than usual were used in each experiment so that a proportionally small movement into one side could more easily be designated significant.

Two other experiments, one with *Sagitta* sp. and the other with small medusae, are, for the sake of convenience, included in this table. No significant migration was apparent in either of them.

Calanus finmarchicus

Forty-seven experiments were performed with *Calanus finmarchicus* and various diatoms and flagellates. These, as Table XVI shows, gave very variable results. The twenty-eight with diatoms gave four positive results (all with starved animals) and three negative (one with fed and two with starved animals) and the eleven with non-toxic flagellates three positive results (two with fed and one with starved animals); the remainder being not significant. This gives a total distribution of seven positive to twenty-nine not significant to three negative.

The results with toxic flagellates were perhaps more consistent—two negative and six not significant out of a total of eight; but considering the series with *Calanus* as a whole it would seem unwise to place any reliance upon them. Ten control experiments and one each with bacteria, oxygen and carbon dioxide all gave not significant results.

TABLE XV. SMALL COPEPODS, ETC. AND DIATOMS

		Copepoda		
		Nitzschia closterium		
H 3	800	—	+ve	*Eurytemora hirundoides*
P 68	2920	S	+ve	—
65	2840	S	+ve	*Tigriopus fulvus*
		Biddulphia sinensis		
P 72	198/ml.	S	+ve	—
78	860/ml.	F	+ve	*Tigriopus fulvus*
		Thalassiosira gravida		
P 73	10	S	+ve	—
		Chaetognatha		
		Hemiselmis rufescens		
P 18	1080	F	N.S.	*Sagitta* sp.
		Small Medusae		
		Nitzschia closterium		
P 6	800	S	N.S.	

Summary distribution of results with small copepods and diatoms.

	+ve	N.S.	−ve
Fed	1	—	—
Starved	5	—	—

The most reasonable deduction to be made is that *Calanus* shows no significant migration in relation to the various phytoplankton organisms used, and that such departures from an even distribution as occur are the result of some swarming or shoaling behaviour and are independent of the distribution of phytoplankton in the tube.

THE VERTICAL APPARATUS

Most of the experiments using the vertical apparatus were done with the copepod *Calanus finmarchicus*. The first ten were performed in the open tank with the apparatus in its original form and these are considered first.

In each experiment the water in one of the tubes had previously been drawn, by siphoning, through a Berkefeld-plaster filter-candle; and the water in the other tube, after similar filtration, had been enriched with one

TABLE XVI. *CALANUS FINMARCHICUS* AND DIATOMS AND FLAGELLATES

Nitzschia closterium

P 51	3440	F	N.S.*
P 12	3060	F	N.S.
P 23	1900	F	N.S.*
P 5	500	F	N.S.
P 1	970	S	+ve
P 2	970	S	N.S.
P 3	970	S	N.S.
P 7	490	S	+ve
P 8	490	S	N.S.

* Medium *Nitzschia*.

Thalassiosira nordenskiöldii

P 58	20	F	N.S.
P 53	18	F	N.S.
P 54	18	F	N.S.
M 3	13	S	−ve
M 4	12	S	N.S.
P 67	10½	S	+ve

Biddulphia sinensis

P 57	222	F	N.S.
P 55	186	F	−ve
P 56	223	S	N.S.
P 52	39	S	N.S.
P 70	—*	S	+ve

* Culture used previously as food for *H. lamornae*.

Skeletonema costatum

P 10	23	F	N.S.
P 9	23	S	N.S.
P 11	23	S	−ve
P 15	Filtered	S	N.S.

Coscinodiscus concinnus

P 59	132	F	N.S.
P 45	84	F	N.S.
P 49	42	F	N.S.

Mixed diatoms

P 36	64	F	N.S.

Oxyrrhis marina, Rhodomonas sp. *and Nitzschia closterium*

P 32	22	F	N.S.
P 35	9	F	N.S.
P 28	4	F	N.S.
P 34	Filtered	F	−ve
P 31	22	S	N.S.
2P 13	3	S	−ve

Dicrateria inornata

2P 8	3220	F	N.S.
P 22	2060	F	+ve
P 4	3900	S	N.S.*
P 17	850	S	N.S.

* 2 rds −ve.

Phaeocystis pouchetii

P 50	—*	F	N.S.
P 40	51	S	+ve
P 37	35	S	N.S.

* Old disintegrating culture.

Cryptomonad

P 46	110	S	N.S.
P 47	110	S	N.S.

Gymnodinium sp.

P 48	120	F	N.S.*
P 39	75	F	N.S.

* 3 rds +ve.

Hemiselmis rufescens

P 14	1168	F	+ve

Nitzschia sp. and a flagellate

P 71	286	F	N.S.

Bacteria

P 24	—	F	N.S.

Oxygen

P 44	—	F	N.S.

Carbon dioxide

P 41	—	S	N.S.

Summary distribution of results with *C. finmarchicus*

Diatoms

	+ve	N.S.	−ve
Fed	—	13	1
Starved	4	8	2

Non-toxic flagellates

	+ve	N.S.	−ve
Fed	2	3	—
Starved	1	5	—

Toxic flagellates

	+ve	N.S.	−ve
Fed	—	5	1
Starved	—	1	1

of a variety of phytoplankton cultures. Twice, exactly 100 animals were picked out and counted before use, but otherwise only roughly equal numbers were put in and then accurately counted after the experiment. The number of *Calanus* swimming in the top compartment of each tube was counted at various irregularly spaced times during the day and night; each experiment lasting about 24 hr. Because of this irregularity it was decided to assess the results on, and apply the test of significance to, the mean of all the readings in each experiment. The percentages calculated from the mean number of animals swimming up in each experiment are given in Table XVII. It will be seen that these figures are small and variable, but that, in all experiments but two, there are more animals swimming up in the enriched water than in the filtered water. If the test of independence described earlier is applied to each experiment, in none is the difference greater than might be expected by chance, but if it is applied to the pooled results, then the difference in behaviour between the two sets of tubes is clearly seen to be significant at the 5% level ($P=0{\cdot}03$). The test for homogeneity confirms that this pooled result is reliable ($P=0{\cdot}85$) and is in fact made up of a series of small and almost uniform differences all in the same direction.

TABLE XVII. RESULTS OF FIRST TEN EXPERIMENTS OUT-OF-DOORS WITH *CALANUS FINMARCHICUS* IN THE VERTICAL APPARATUS

Serial no.	Phytoplankton organism	No. of animals		Total mean % swimming up	
		Contl.	Exptl.	Contl.	Exptl.
ML 16	Mixed diatoms	58	60	0·2	1·5
ML 17	Mixed diatoms and flagellates	217	226	4·0	3·9
ML 23	Mixed diatoms and flagellates	55	59	1·3	3·8
ML 25	*Syracosphaera carterae*	73	82	0·3	3·6
ML 29	*Ditylum brightwellii*	100	100	0·8	4·4
ML 30	*Gymnodinium* sp.	99	119	1·2	5·5
ML 33	Water rich in *Mesodinium**	100	100	0·9	1·9
ML 34	*Peridinium trochoidium*	84	97	1·0	2·8
ML 36	*P. trochoidium* and *Gymnodinium* sp.	142	218	2·8	2·7
ML 37	*P. trochoidium* and *Gymnodinium* sp.	215	270	0·5	1·2

A summary of the χ^2 results confirming that these differences are significant is as follows:

	Degrees of freedom	χ^2	P
Interaction	9	4·98	0·85
Pooled readings	1	5·0	*ca.* 0·03
Total of individuals readings	10	9·98	

* This water was unfiltered and as taken from the sea during a rich outburst of the ciliate *Mesodinium* sp.

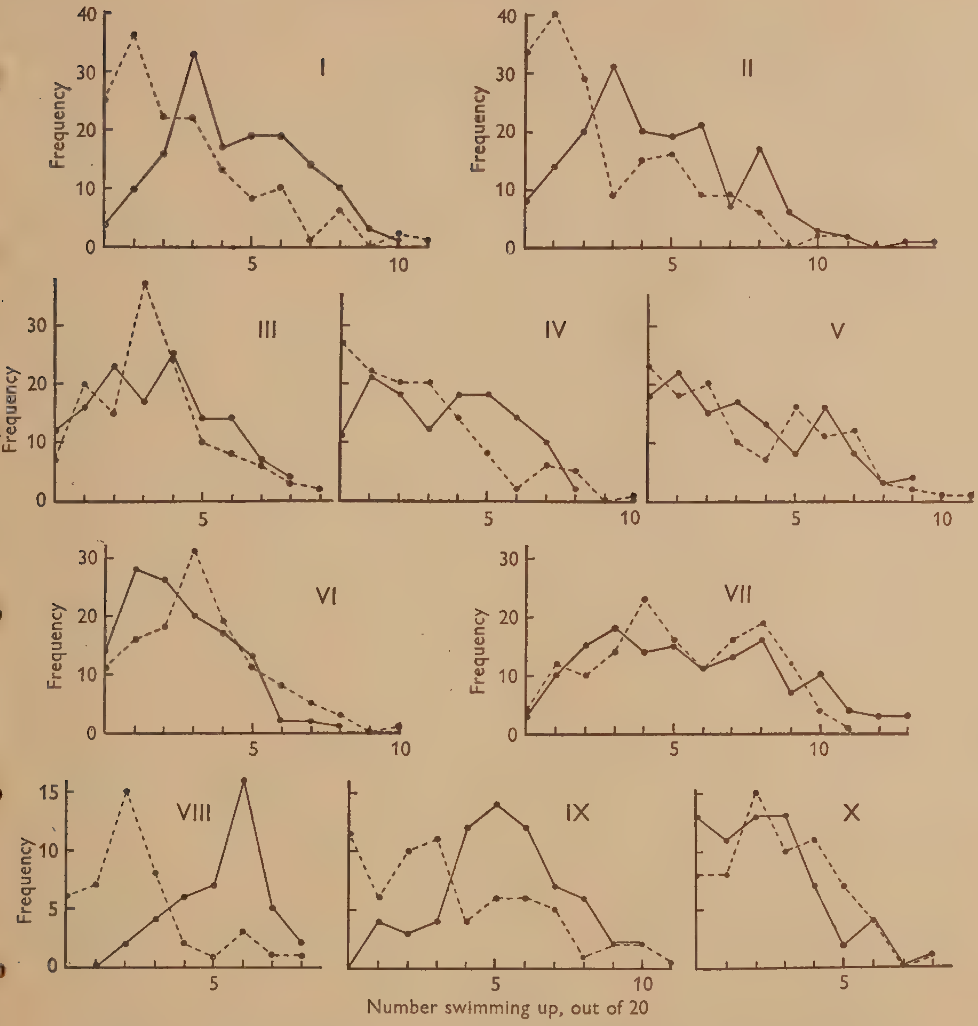
As the reaction of the animals to all these different phytoplankton organisms appears to be similar, it may be taken as permissible to calculate a total mean percentage of animals swimming up for the whole series of experiments. If this is done, 1·3% are found to swim up in the filtered water and 3·1% in the enriched.

After these preliminary experiments 103 were performed with the apparatus indoors in the aquarium tank. This whole series is more uniform in that readings were taken at regular intervals after the start of the experiment, and the same number of counted animals was used each time. The fact that each experiment involved two control and two experimental tubes gave a further check on the reliability of the results. The whole series falls into ten groups dependent upon the phytoplankton organisms used and may best be considered in this manner. Detail of the method of presentation of the results is given in the first group.

Group I: *Skeletonema costatum*

Ten experiments were performed with the pair of control tubes containing water filtered by suction through a Whatman no. 1 filter-paper and aerated before use by shaking, and with the experimental tubes containing similar water enriched with a culture of *S. costatum*. A small quantity of tap water was added to the water in the control tubes to make its specific gravity equal to that of the enriched water, the latter being slightly more dilute owing to the addition of the culture. The volume added was calculated, and was of the order of 5–10 ml. depending upon the amount of culture used. Twenty *Calanus finmarchicus* were picked out from a townet catch taken as short a time as possible before the experiment, and put into the top of each tube. The tubes were then suspended in the tank and the number of animals in the top compartment of each was counted from time to time. In order to economize in the use of cultures it was sometimes decided, especially when an experiment was of short duration, to use the water more than once. On these occasions it was poured out, filtered through coarse silk to remove the animals, and replaced in the tubes ready for use with a fresh sample of *Calanus*.

A satisfactory way of showing pictorially whether there was any difference in behaviour between the pairs of tubes was found to be as follows. The frequency with which each particular reading occurs throughout the whole group of experiments is determined, and this number plotted against the value of the reading. The result for all the experiments involving *Skeletonema costatum* is shown in Text-fig. 3 (I). The ten experiments gave a total of seventy-three readings, each in pairs (there being two control and two experimental tubes), and this provides 146 values for filtered water and 146 for enriched. It can be seen at once that there are more of the higher readings in the enriched water and in fact the curve for the latter bears a close resemblance to that for filtered water but is moved bodily to the right.



Text-fig. 3. Upward movement of *Calanus* in water containing various organisms, each graph representing the results of a series of comparable experiments with the organism stated. Each graph plots the frequencies of the different values, out of twenty, for the number of animals moving up—either in water rich in the experimental culture (continuous line), or in a control medium (broken line). The control was usually filtered sea water, but enriched water was chosen for series III, VI and VII. I, *Skeletonema costatum*; II, *Ditylum brightwellii*; III, *Gymnodinium* I; IV, *Chlamydomonas* sp.; V, Flagellate One; VI, *Gymnodinium* II; VII, *Oxyrrhis*, *Rhodomonas*, and *Nitzschia*; VIII, mixed phytoplankton; IX, *Coscinodiscus concinnus*; X, *Chlorella stigmatophora*. See Tables XVIII and XIX.

TABLE XVIII. SUMMARY OF VERTICAL RESULTS WITH *CALANUS FINMARCHICUS*

		Mean % swimming up		
	Series	On 60 min. readings	On 1st four readings	On all readings
I.	Control-filtered	16·0	16·4	10·5
	Skeletonema costatum	27·8	23·9	17·5
II.	Control-filtered	21·7	22·4	14·0
	Ditylum brightwellii	29·5	29·2	22·5
III.	Control-enriched	20·0	18·8	16·6
	Gymnodinium I	20·2	18·9	17·4
IV.	Control-filtered	14·4	14·8	12·9
	Chlamydomonas sp.	20·2	19·3	16·9
V.	Control-filtered	17·5	18·2	16·5
	Flagellate One	17·2	18·1	16·3
VI.	Control-enriched	15·9	14·2	16·4
	Gymnodinium II	13·6	11·5	12·5
VII.	Control-enriched	29·7	27·9	25·5
	Oxyrrhis marina etc.	30·7	29·1	27·9
VIII.	Control-filtered	15·0	15·0	12·2
	Mixed phytoplankton	30·8	29·6	26·6
IX.	Control-filtered	23·0	20·8	22·2
	Coscinodiscus concinnus	36·0	29·1	26·3
X.	Control-filtered	15·5	14·8	14·0
	Chlorella stigmatophora	11·5	13·1	11·4

TABLE XIX. SIGNIFICANCE TESTS FOR DATA PRESENTED IN TABLE XVIII AND TEXT-FIG. 3

(These test whether observed differences in the number of *Calanus* swimming up (in the vertical apparatus) are significant. Calculation from 60 min. reading for all experiments (column 1 in Table XVIII), in each series.)

	Series		Degrees of freedom	χ^2	*P*
I.	*Skeletonema costatum*	Interaction	19	22·10	*ca.* 0·25
		Pooled	1	16·19	<0·001
		Total	20	38·29	
II.	*Ditylum brightwellii*	Interaction	19	15·88	0·7
		Pooled	1	6·73	0·01
		Total	20	22·61	
IV.	*Chlamydomonas* sp.	Interaction	23	31·97	>0·10
		Pooled	1	5·65	<0·02
		Total	24	37·62	
VI.	*Gymnodinium* II	Pooled	1	0·95	*ca.* 0·35
VIII.	Mixed phytoplankton	Interaction	5	1·69	*ca.* 0·9
		Pooled	1	8·51	<0·01
		Total	6	10·20	
IX.	*Coscinodiscus concinnus*	Interaction	9	13·65	0·15
		Pooled	1	8·12	<0·01
		Total	10	21·77	
X.	*Chlorella stigmatophora*	Pooled	1	1·37	*ca.* 0·25

To test whether the difference is significant, the test of independence can be applied either to one reading from each particular experiment or to the mean of a group or the mean of all the readings from each particular experiment. Examination of the results for one or two of the experiments revealed that the number of animals swimming up generally rose to a maximum at about 1–1½ hr. and then fell gradually for the rest of the time. It was decided consequently to apply the test to the reading taken at, or as close as possible to, 1 hr. after the start of the experiment and to use only this one reading from each experiment. When this is done for the *Skeletonema* results it is found that only four out of the total of twenty show significant differences. (The test is applied twice to each reading; each time one control tube being compared with one experimental tube, and this results in twenty values of χ^2 for the ten experiments.) When, however, the single readings are lumped together and the test applied to this pooled result the whole series is shown clearly to be significant and, further, satisfies the test for homogeneity. A summary of the values of χ^2 concerned is given in Table XIX.

It may therefore be concluded that a significantly greater number of *Calanus* swim up in the enriched tubes than in the filtered tubes. The total mean percentage swimming up, as calculated from the 60 min. readings, may be taken as a measure of the actual difference between the two tubes. This is 16·0% for the filtered water and 27·8% for the enriched. The average of the concentrations of cells in the enriched water used in the various experiments is 18·3 cells of *Skeletonema costatum* per mm.^{3}. This figure includes both single cells and cells in chains of various numbers.

Group II. *Ditylum brightwellii*

A series of experiments precisely similar to the above but using the diatom *Ditylum brightwellii* gave similar results. The graph in Text-fig. 3 (II) reveals a similar displacement of the curve for enriched water and that for the filtered water is almost identical with the one in the previous series. There is perhaps a little more irregularity in the enriched curve, and the whole is more flattened with more readings of higher value and fewer of more intermediate value. Only one of the twenty values of χ^2 calculated from the 60 min. readings is significant of itself, but the pooled result is clearly significant and the test for homogeneity is easily satisfied (Table XX). In fact there is less variation within this series than in the previous. A summary of the χ^2 values is given.

Significantly more *Calanus* therefore swim up in the *Ditylum*-enriched water. The total mean percentages swimming up being 21·7% in the filtered water and 29·5% in the enriched: both values a little higher than those with *Skeletonema*. The average concentration of *Ditylum* in the enriched water used was 527·3 cells/ml. or 0·53/mm.^{3}.

Group III. *Gymnodinium* I

Evidence of the effect of the two diatoms having been found, *Gymnodinium* I, one of the organisms suspected of having a toxic effect, was next tried. With the possibility, after the hypothesis of animal exclusion, that *Gymnodinium* might suppress vertical migration to some extent, the control chosen for this series was an enriched water and not a filtered one. Twelve experiments of this nature were performed. The water was first passed through a Whatman no. 1 paper and one lot was enriched with the *Gymnodinium* culture and the other variously with *Skeletonema*, *Chlamydomonas* and a culture of mixed phytoplankton organisms grown by seeding the normal culture solution with a little raw sea water taken at the time of a diatom outburst. This culture contained diatoms and flagellates of several kinds including *Skeletonema*, *Thalassiosira* and a *Nitzschia*.

As may be seen from the graph in Text-fig. 3 (III), behaviour of the *Calanus* in the two sets of tubes was almost identical and closely resembled that in the enriched water in the two previous series. No tests of significance were applied and the total mean percentage swimming up in the enriched control was 20·0% and in the *Gymnodinium*-enriched water 20·2%. The average concentration in the latter was 9·8 *Gymnodinium* cells/mm.^{3}: and in the former 47·4 cells/mm.^{3} of various kinds. It may be noted that the percentages swimming up are rather smaller than in the previous experiments.

Group IV. *Chlamydomonas* sp.

This series consisted of twelve experiments having filtered water in the control tube and water enriched with *Chlamydomonas* sp. culture in the experimental tube. Examination of the graph in Text-fig. 3 (IV) reveals the likelihood of some difference in behaviour between the two tubes, but this is by no means so clear as in the previous experiments. The control curve is similar in form to that obtained before, although it is somewhat flattened. Altogether there are sixty-two pairs of readings giving 124 values for each type of water. This is less than the numbers in groups I and II and may partly account for the difference in shape. The enriched curve lacks the definite peak characteristic of the previous curves, but nevertheless indicates a preponderance of higher readings.

When the test of independence is applied to the 60 min. readings, four out of the twenty-four are shown to be significant, but one of these has the greater number swimming up in the control tube. Despite this, when the results are pooled and the test applied to the totals, their difference is shown clearly to be significant (Table XIX). The test for homogeneity is just satisfied and hence it is possible to place reliance on this result. The readings are, however, very variable and the difference in behaviour is neither as clearly marked nor as great as in the diatom experiments. The mean percentage swimming up in the control tubes was 14·4% and that in the enriched tubes 20·2%. The average concentration of *Chlamydomonas* employed was 73·7 cells/mm.^{3}.

Group V. Flagellate One

Ten experiments were performed using water enriched with a culture of Flagellate One. This is one of the organisms which the toxicity experiments indicated might be harmful, but it was still compared with a control of Whatman no. 1 paper-filtered water. The total number of pairs of readings is sixty-two, this being equal to the number in the previous group. Inspection of the graph in Text-fig. 3 (V) reveals almost complete identity of behaviour between the two lots of water. Further, both curves closely resemble the control in the previous group, being only slightly flattened and having a few more readings of higher value. The mean percentages swimming up are 17·5% in the control and 17·2% in the enriched tubes. As a result of this no test for significance was applied. The average concentration of Flagellate One used in the first eight experiments was 94 cells/mm.^{3}. In case this culture was not sufficiently dense to manifest any possible 'exclusion' effect the remaining two experiments were performed at a concentration of 209 cells/mm.^{3}. There was, however, no apparent difference in behaviour, and it may be concluded that the presence of Flagellate One in no way affected the number of *Calanus* swimming up. The average concentration of cells employed in the whole series was 117 cells/mm.^{3}.

Group VI. *Gymnodinium* II

Eleven experiments were performed with *Gymnodinium* II, another of the flagellates shown to be toxic. The chosen control was water enriched with a mixed phytoplankton culture, grown, as before, by seeding with raw sea water. This culture consisted of almost pure *Nitzschia closterium* of the normal large form with a little *Skeletonema* and a few flagellates. In both the control and the experimental tubes the water was paper-filtered before being enriched. In one of the experiments only one pair of the four tubes was used, and as a result there are fifty-nine pairs of readings and five odd ones giving a total of 123 values each for the control and the experimental curves. Examination of the graph in Text-fig. 3 (VI) shows the form of the enriched control curve to be very similar to those obtained previously, including again the characteristic peak. The experimental *Gymnodinium* curve is also similar but is displaced now to the left and almost in the position of previous control curves. Two of the twenty-one values of χ^2 show the difference between the two tubes to be significant. When the 60 min. readings are pooled, however, the χ^2 value does not even approach that required for significance (Table XIX). It seems therefore that, although fewer animals swim up in the *Gymnodinium* II enriched water, not enough stay down to give a significant difference, and the effect must be ascribed to chance. The total mean percentage swimming up in the diatom-enriched control was 15·9%, and that in the *Gymnodinium*-enriched 13·6%. The average concentration in the former was 5·6 cells/mm.^{3} and in the latter 9·5 cells/mm.^{3}.

The mean percentages swimming up are later calculated on the first four readings in every experiment and also on all the readings. Examination of Table XVIII suggests that, with *Gymnodinium* II, the difference between the two tubes increases with time. The pooled χ^2 test was therefore applied both to the means of the first four readings and to the means of all the readings in each experiment, but neither of the values obtained showed a significant difference, although they were higher than for the 60 min. readings.

Group VII. *Oxyrrhis marina*, *Rhodomonas* sp. and *Nitzschia closterium*

Ten experiments were performed with the mixed culture of *Oxyrrhis*, *Rhodomonas* and *Nitzschia*, the *Rhodomonas* component of which is now thought to be toxic. The control was again chosen to be water enriched with mixed phytoplankton. This mixed culture consisted chiefly of *Skeletonema*, a naviculoid collected in chains, and *Thalassiosira*, with a little *Chaetoceros* and some flagellates. Altogether, seventy-one pairs of readings were taken giving 142 values for each of the cultures. The graph in Text-fig. 3 (VII) shows the behaviour of the animals in the two sets of tubes to have been almost identical. Both curves are typically those of enriched water but they lack the peak which is common in most of the earlier experiments and also include a greater number of higher values than previously. The curious dip in the centre of both curves at the value six is interesting. The total mean percentage swimming up in the enriched control was 29·7% and that in the *Oxyrrhis*-enriched water 30·7%. No tests were applied. The average concentration of diatoms in the enriched control water was 0·74 chain/mm.^{3}, a chain of average length being about four cells, together with some single cells. The concentration in the mixed experimental water was 6·4 cells of *Oxyrrhis*/mm.^{3}, together with a few *Nitzschia* and a very few *Rhodomonas* cells. This fact may account in part for the absence of any depressant action on the vertical migration of the *Calanus*.

Group VIII. Mixed phytoplankton organisms

No clear difference having been found between filtered and enriched water in the last four series, it was considered desirable to confirm that this difference could still be demonstrated. Consequently, three experiments were performed with Whatman no. 1 paper-filtered water in the control tubes and similar water enriched with a mixed phytoplankton culture in the experimental tubes. This culture was grown as before, but was rather older and contained the normal large *Nitzschia closterium*, various small and large flagellates, a heliozoan and a large ciliate. Twenty-two readings were taken, giving forty-four values for each of the curves. Because of this low number the graph in Text-fig. 3 (VIII) is drawn to double the vertical scale used previously. A difference greater than any so far obtained between behaviour in the two waters is immediately apparent; and both curves closely resemble the early ones despite the higher numbers of animals swimming up. Even with so small a number of experiments the pooled 60 min. results, when the test of independence is applied, are seen clearly to be significant (Table XIX). Although none of the six single 60 min. readings are significant of themselves, the test for homogeneity shows them to be more uniform than any of the other series and the pooled result is therefore reliable. The total mean percentage swimming up in the filtered water was 15·0% and that in the enriched 30·8%. The concentration of phytoplankton in the enriched water was 2 cells/mm.^{3}.

Group IX. *Coscinodiscus concinnus*

The next five experiments used a culture of the large diatom *C. concinnus*. The control tubes contained Whatman no. 1 paper-filtered water and the experimental tubes water enriched with the *Coscinodiscus* culture. Twenty *Calanus* were again put into each tube. Thirty-three readings were made giving sixty-six values for each curve, and the graph in Text-fig. 3 (IX) is once more drawn to double the vertical scale. A preponderance of higher readings in the enriched water is again apparent, although the curve has a less pronounced and more rounded peak. This, however, accords well with the more flattened nature of the control curve (deriving from the greater number of higher readings) which, with a little extra irregularity to be expected from the smaller number of readings, otherwise closely resembles the early ones. It is particularly like the group IV and V control curves. Application of the test of independence to the 60 min. readings gives three out of the ten as significant, but when it is applied to the pooled figures the whole series is shown to be so (Table XIX). The homogeneity test is satisfied although there is more variation between different readings than in series VIII. The total mean percentage swimming up in the control tubes was 23·0% and that in the enriched ones 36·0%. Both these figures are the highest obtained for these two types of water in all the ten groups of experiments. The average concentration of *Coscinodiscus* in the enriched water was 22·4 cells/ml. or 0·02/mm.^{3}.

Group X. *Chlorella stigmatophora*

The last five experiments with *Calanus* utilized a culture of the non-motile alga *Chlorella stigmatophora* which has been shown to have toxic properties. The control chosen was paper-filtered water and thirty-two pairs of readings were taken, the graph in Text-fig. 3 (X) being drawn to the same scale as the previous two. The control curve is seen to be steeper and more like the first obtained, while the curve for the enriched water is very similar. The total mean percentages swimming up are 15·5% in the filtered water and 11·5%

in the enriched, suggesting the possibility of a significant difference in behaviour between the two. This is not verified, however, and the test of independence demonstrates significance neither on the individual 60 min. readings nor on the pooled figures. The average concentration of *Chlorella* in the enriched water was 226 cells/mm.^{3}. This is well below even the optimum found in the toxicity experiments and need not necessarily have any harmful properties.

The summary of the results in Table XVIII shows the mean percentages swimming up as calculated from (*a*) the 60 min. reading from each experiment, (*b*) the mean of the first four readings, and (*c*) that of all the readings in each experiment. It can be seen that, generally, in each series as time goes on fewer animals swim up. This gradual decline in the proportion swimming up has been mentioned earlier as the reason for using the 60 min. readings in assessing the significance and magnitude of the differences. It also emphasizes the importance of using animals caught as short a time as possible previous to the experiment, and it must probably be attributed to a general change in behaviour on being kept in the unnatural conditions of captivity.

The second change with time to be seen is that between successive series of experiments. There are considerable differences between what should be comparable figures in the different series. There are altogether seven series with filtered controls, and the proportions swimming up in these range from 23·0% in IX to 14·4% in IV. These particular experiments cover a period of just over 2 months and during that time, of course, considerable changes were taking place in the population of *Calanus* which was drawn on for the experimental animals. This factor, combined with the varying light and temperature conditions, probably accounts for this variation with time. Male, female, and stage V and occasionally stage IV *Calanus* were used just as taken in the townets; the plan being to test the reactions of a typical sample and not one particular stage. The stages and sexes present were determined from time to time, but the type of behaviour obtained could not be correlated with the composition of the sample. On account of this fluctuation with time it is perhaps unwise to make comparisons between the effects of different cultures. However, taking the percentage differences between the filtered control and the enriched experimental tubes in each of seven series, and the enriched control and experimental tubes in the other three, and arranging the figures in order of magnitude we obtain the scheme given in Table XX.

Each of the two columns in itself may be reliable in showing the relative degree to which the various cultures affect the number of *Calanus* swimming up. Relation of the one column to the other, however, presents difficulties. There are two alternatives. If the behaviour towards the enriched control water in each of the *Gymnodinium* I and II and the *Oxyrrhis* experiments is taken as identical with that in the mixed-phytoplankton-enriched experimental water in column I (i.e. the top value of 16); then the three cultures should be assigned the positive values of 16, 15 and 14 as shown in brackets (i.e. 16 minus 0, 1 and 2 respectively). This puts them at the top of the whole series as regards degree of effect on *Calanus*. If, however, the differences invoked by the first five (significant) cultures in column I are averaged and this figure of II is taken as the common denominator, then the three cultures must be assigned the values of +11, 10 and 9 as underlined, and they should then be placed between *Skeletonema* and *Ditylum* in the whole series.

TABLE XX. COMPARISON OF THE EFFECTS OF DIFFERENT CULTURES IN THE VERTICAL APPARATUS

	% differences from filtered water		% differences from enriched water			
S	Mixed phytoplankton	16	*Gymnodinium* I	0	(+16)*	+11†
			Oxyrrhis	−1	(+15)*	+10†
			Gymnodinium II	−2	(+14)*	+9†
S	*Coscinodiscus*	13				
S	*Skeletonema*	12				
S	*Ditylum*	8				
S	*Chlamydomonas*	6				
	Flagellate One	0				
	Chlorella stigmatophora	−4				

( ) * Equating enriched control with mixed phytoplankton result.
— † Equating enriched control with mean of significant phytoplankton results.
S indicates those differences shown mathematically to be significant.

It is reasonable to suppose that different phytoplankton organisms may affect the behaviour of *Calanus* differently, but if this should be so it is not possible, with only the present evidence available, to state with any certainty the relative degree to which they act. It is clear, however, that more *Calanus* swam up in the presence of all of the phytoplankton organisms tested, except Flagellate One and *Chlorella stigmatophora*.

Besides the above series of experiments with *Calanus*, three each were done with decapod larvae and young *Thysanoessa inermis*. All but one of these were with a mixed phytoplankton culture similar to that used in group VIII above, the other being with *Chlamydomonas*. The control for each was Whatman no. 1 paper-filtered water. No significant difference in behaviour between the pairs of tubes could be detected in any of the experiments, either when tested individually, or when pooled. The number of animals swimming up was greater with the decapod larvae, but this derives from their marked positive reaction towards light. The total mean percentages swimming up in the three decapod larva experiments were 70·0% in the filtered control and 71·5% in the enriched water. In the three *Thysanoessa* experiments there were 17·9% in the control and 19·3% in the enriched water. Details of the individual results are given in Table XXI.

Finally, five experiments were done using ten young *Hemimysis lamornae* in each tube of the apparatus in its original form. Owing to the marked movement of *Hemimysis* away from light these experiments were done in complete darkness in a constant temperature room and the animals counted from time to time by aid of a dim red lamp. All had ultra-filtered water in the control tube, and in the experimental tube similar water enriched in three experiments with *Chlamydomonas* and in two with *Skeletonema* culture. The results have been assessed on the mean of all the readings in each experiment. When this is done no individual experiment is shown by the test of independence to be significant; but if all the results are pooled, the series is clearly so, and the test for homogeneity is satisfied (see Table XXI). The total mean percentages swimming up were 13·8% in the filtered and 32·0% in the enriched tubes. The average concentration of *Chlamydomonas* in the enriched water of the first three experiments was 93 cells/mm.^{3} and of *Skeletonema* in the last two, 26 cells/mm.^{3}.

TABLE XXI. RESULTS OF EXPERIMENTS WITH *CARCINUS* ZOEAS *THYSANOESSA* AND *HEMIMYSIS* IN THE VERTICAL APPARATUS

	Mean % swimming up	
	Control	Experi-mental
20 *Carcinus maenas* zoeas in each tube:		
Mixed phytoplankton 0·84 chains/mm.^{3}	50·8	63·3
Mixed phytoplankton 0·84 chains/mm.^{3}	62·8	64·4
Chlamydomonas sp. 99 cells/mm.^{3}	84·6	83·4
20 young *Thysanoessa inermis* in each tube:		
Mixed phytoplankton 3 cells/mm.^{3}	29·2	29·5
Mixed phytoplankton 3·4 cells/mm.^{3}	10·7	13·7
Mixed phytoplankton 4·4 cells/mm.^{3}	16·0	13·2
20 young *Hemimysis lamornae* in each tube:		
Chlamydomonas sp. 90 cells/mm.^{3}	6·1	20·8
Chlamydomonas sp. 92 cells/mm.^{3}	9·0	26·0
Chlamydomonas sp. 96 cells/mm.^{3}	3·0	25·0
Skeletonema costatum 26 cells/mm.^{3}	26·0	30·0
Skeletonema costatum 26 cells/mm.^{3}	25·0	60·0

Summary of χ^2 tests for *Hemimysis*:

	Degrees of freedom	χ^2	*P*
Interaction	4	1·57	*ca.* 0·8
Pooled readings	1	4·70	0·04
Total	5	6·27	

UNDERWATER OBSERVATIONS

If the results of experimental work of the kind reported here are to be applied to a consideration of problems in the sea itself, it is important to know whether or not the behaviour of the animals in the various pieces of apparatus used resembles their behaviour under natural conditions. Underwater observations on the behaviour of some of the animals used have already been described (Bainbridge, 1952). Ten more excursions were made in 1952, each involving descents in different places and giving a total of about 8 hr. underwater. In particular, the technique of swimming with foot-flippers and following animals while over deep water has been developed successfully. All the 1951 observations on the spring brood of *Calanus* have been verified, although the 'upper zone' of 12 in. has seemed to be very much shallower this year, and there have been fewer animals in the deeper 'zone of migration'. In particular, the marked cessation of motion when the sun is covered has been noticed repeatedly. The whole sea gives a peculiar impression of idleness on such occasions. Besides the almost universal vertical swimming up and down, a more erratic movement has been seen once or twice. This is distinct from the bouncing along on the under surface of the water (which has also been seen again) and results in little or no forward movement at all. It is a violent dashing about in different directions, and is so rapid that it is not possible to tell how the animal is orientated or what limbs are being used. It is perhaps most adequately described as a 'caper' and seems to be rare. On one occasion also *Calanus* was seen swimming horizontally, deep in the body of the water with an uncommon sort of jerky side-to-side motion. Apart from these exceptions the continued occurrence of straight up and down swimming makes it certain that this is the form generally indulged in. It should perhaps again be noted that the downward swimming is invariably head-first down. Some *Calanus* from a deep townetting, when taken below in a jar and released, swam head-first downwards out of sight.

The collection in small swarms or clusters was again noticed, although these appeared smaller this year with rarely more than six animals in a group. One stage V *Calanus* was seen swimming with three adults, all circling round and round each other.

There were again many small copepods and young stages, but these were harder to observe because of the large amount of detritus repeatedly found in the water. A random sort of motion was prevalent, and even those swimming upwards appeared to do so in a jerky and 'kinetic' manner and then bounce away similarly (often towards the sun) along the under surface of the water. None were ever seen to go downwards in this way and it may perhaps be assumed that they gradually sink downwards to go jerkily up to the surface again after a certain time. Many seemed to hang head-first downwards but it was not possible to decide whether they sank in that position or whether they were preparing for swimming down.

Perhaps the most interesting observations concern three decapod larvae. The first two of these were megalopas both seen on 23 May. They were pale in colour and were rather small and were both swimming rapidly in a perfectly horizontal straight line, one about 2 ft. below the surface and the other about

3 ft. I was able to follow one for about 12 ft. and the other for 24 ft. There was no sign of deviation whatsoever, and the movement appeared most purposeful. They were both swimming roughly at 20° to the right of the direction of the sun's rays. The third was a zoea seen on 3 July. This was large and probably *Carcinus*. It was about 4 or 5 ft. down and was swimming almost horizontally (slightly upwards) in a gently undulating line. (It is just possible that the undulation was the effect of my own movements, but this is very unlikely.) These three are the only decapod larvae so far seen.

Three young *Thysanoessa inermis* caught several days previously and used in one of the experiments were taken down and released separately from small jars. They all went down, one sinking, one swimming quite vigorously obliquely, and one head-first downwards.

Many ctenophores and medusae were seen on different occasions and these were usually quite randomly orientated. One large *Bolinopsis* was seen travelling horizontally, its large oral lobes streaming behind. Very many, roughly spherical, transparent bodies with six to ten small bubbles enmeshed in them were seen drifting along during one descent. One was taken in a breffit and proved to be *Oikopleura* in its house. Examined later in the laboratory, the house was found swarming with flagellates of all kinds and a few small diatoms and ceratia, etc. It seems likely that this enormous concentration of even the smallest flagellates was producing the tiny oxygen bubbles by photosynthesis. These must have buoyed up the house and affected the animal's movements in some way.

One descent was made purposely during rough weather. There was a great amount of wave action resulting in swirling about of medusae, ctenophores and a few small copepods. This movement went down to about 10 ft. but diminished gradually. It was possible to follow some of the animals, but they were all being swirled about the whole time and no directive movement was observed.

GENERAL DISCUSSION

Experiments in the Horizontal Apparatus

Considering first the results obtained with the horizontal apparatus it may safely be claimed that, under the conditions of the experiments, significant migrations by various animals into concentrations of phytoplankton have been demonstrated. This is particularly so with the mysids used, which swam into water enriched with cultures of *Skeletonema*, *Thalassiosira*, *Biddulphia*, *Nitzschia* and mixed diatoms and into cultures of the flagellates *Chlamydomonas*, *Peridinium*, *Dicrateria*, Flagellate K, and *Oxyrrhis*. No marked movement into the diatoms *Lauderia*, *Coscinodiscus* and *Eucampia* was observed nor into the flagellates *Syracosphaera* and *Exuviaella*, but a movement away from *Rhodomonas* and *Gymnodinium* II was demonstrated. Various small copepods swam into cultures of the diatoms *Nitzschia*, *Biddulphia* and *Thalassiosira*, and positive results were also obtained with *Artemia salina*. It was not possible, however, to obtain consistent or intelligible results with either *Calanus finmarchicus* or with various decapod larvae.

There can be little doubt that these positive migrations are for the purpose of feeding; and, although this could not be demonstrated significantly, there was a greater incidence of them amongst starved animals than amongst fed ones. No experiments have so far been designed especially to throw light on the mechanism underlying the movement, but it seems likely that sometimes the effect of the phytoplankton may be mediated by substances dissolved in the water. This would not seem to be either concentration of carbon dioxide (which has the opposite effect), oxygen (which has no determinable effect) or pH (in which property the cultures do not differ appreciably from normal water), but more probably some kind of dissolved organic substance. Sometimes the actual physical presence of the diatoms is necessary.

Those flagellates invoking an avoiding reaction in the animals almost certainly do so through some substance dissolved in the water; this would seem both harmful and distasteful, although the deaths produced by Flagellate 12 seemed to result from the actual ingestion of the cells. The less consistent results with these cultures classed as 'toxic' are perhaps due to there being two conflicting reactions on the part of the animals—an avoidance of the water due to the distasteful substance in it and a movement into the actual concentration of cells for the purposes of feeding. If the feeding reaction is strong, due perhaps to starvation, a positive migration will result, if the avoiding reaction is strong, then a negative migration may. With the bacteria culture, of presumably little nutritive value to those animals tested, a consistent negative migration can be obtained: here clearly away from some dissolved organic substance.

In extent, both the positive and negative migrations seem to indicate an all-or-nothing reaction. The lumping together of those readings in any particular group which are classed as 'not-significant' but which might have been expected to be positive does not give any indication of a positive tendency but rather emphasizes the random nature of the distribution. It thus seems that if a positive or negative migration is to take place it does so without equivocation. In the mysids this may be connected with the shoaling reaction which they exhibit. The marked positive migrations into concentrations of ammonia are of particular interest on this account. Ammonia is one of the excretory products of these animals, and a tendency to congregate in water richer in ammonia would automatically act as an aggregating mechanism for them. Any small group of animals containing individuals circling round and round each other as they do, would excrete and develop its own cloud of ammonia round it. This would attract others and the whole would work on a snow-ball principle, gathering more and more animals into a bigger and bigger cloud. Such a sequence could conceivably take place most quickly in the presence of food and could constitute part of the mechanism behind the migrations in the tubes. Those shoals of mysids seen in aquaria and tanks must almost always be brought into being by uneven lighting conditions, but this cannot generally be so in nature and especially in the open sea. The shoals of euphausids seen by many observers (Hardy & Gunther, 1935; Hart, see below), and the uneven distribution of small plankton animals reported by Barnes & Marshall (1951), and seen underwater by myself, cannot be dependent upon light. There may be many means by which animals keep together in swarms, such as sight or a rheotactic sense, but the possibility of some agent such as ammonia being involved in the mechanism in some species must be borne in mind.

Satisfactory and understandable results were not obtained with *C. finmarchicus* in the horizontal tubes and with the few samples of decapod larvae used. This would seem to be due to the different swimming habits of the various animals. As has been mentioned, it is not generally thought that members of the plankton possess powers of active migration of any consequence in the horizontal plane. The experimental results point to this being so with *Calanus* and it was for this reason that the vertical experiments were developed. This apparently fundamental tendency of *Calanus* to swim in a vertical plane is amply confirmed by the underwater observations.

This is not true of the other animals used in the horizontal tubes. The various mysids and *Artemia* appeared to behave perfectly naturally when indulging in their horizontal excursions. It is unfortunate that comparable animals could not be observed under water. The mysids are mostly nocturnal, when they cannot be seen, and in the daytime the euphausids are restricted to comparatively deep water. Those *Thysanoessa* released from captivity under water swam in straight lines with the long axis of the body pointing forwards but not horizontally, of course, as the initial response was to go away from the higher light intensity.

The decapod larvae that were seen were behaving exactly as might be expected if they are to fit into the group possessing powers of horizontal movement. The very variable results obtained with them are all the more puzzling. They may, of course, be due to the lack of any definite behaviour reaction towards the particular cultures used but they are nevertheless disappointing. It is interesting that six out of the twelve are significant one way or the other.

The surprising results with small copepods, where six out of six experiments showed the most definite movements into diatom cultures, may be accounted for by a third type of swimming behaviour. This is the random movement repeatedly observed under water and it is of course precisely such as would result in the rapid development of an uneven distribution of animals in the tube if there should be a tendency on their part to remain in one particular environment. Seen in the sea it is a vigorous movement, and canalized as it is in the tubes, it could result in quite a rapid horizontal movement. The experiments, nevertheless, demonstrate a marked preference by the animals for water rich in diatoms.

Experiments in the Vertical Apparatus

Considering the results obtained with the vertical apparatus it may again safely be claimed that, under the conditions of the experiments, significantly greater numbers of animals are shown swimming up in the presence of cultures of mixed phytoplankton, *Gymnodinium* I and II, *Oxyrrhis*, *Coscinodiscus*, *Skeletonema*, *Ditylum* and *Chlamydomonas*; while Flagellate One has no effect on the behaviour of *Calanus*, and *Chlorella* appears to depress the numbers swimming up. The control in some of these experiments was ultra-filtered water, but generally it was water filtered through a paper of the normal pore size. This was especially intended to allow minute flagellates and diatoms to pass through but to stop all the larger cells. The behaviour of the animals in the enriched water was therefore being compared with their behaviour in water containing a low concentration of food and not in entirely sterile water such as would not be found in the sea. In all the indoor experiments the intensity of light was such as to allow an average of about 25% of the animals to swim up. Under these conditions roughly twice as many did so in the enriched tubes as in the filtered, but as mentioned earlier some of the phytoplankton organisms used may have a greater effect than others.

The animals in the tubes appeared to resolve themselves into two distinct groups, the one swimming up and the other down. Those in the top compartment were generally in the upper half of this and, when *Calanus*, performed their 'hop-and-sink' movements there. Those in the lower compartment were generally in the lower half and some would be swimming head-first downwards and others doing the 'hop-and-sink'. It seems likely, therefore, that the short length of the tubes does not greatly affect the manifestation in the population they contain, of these two groups. There was, of course, interchange between the two but this was usually fairly rapid. In general, the swimming behaviour, especially of *Calanus*, bore a close resemblance to that seen in the sea.

The five experiments with young *Hemimysis lamornae* gave much greater numbers swimming up in the presence of *Chlamydomonas* and *Skeletonema*. Such young forms in nature are often found higher in the water and might be expected to show this sort of vertical movement in the tubes. The results with decapod larvae and *Thysanoessa* are more puzzling. The latter are very delicate and proved difficult animals from the experimental point of view. They are caught only in deep water and do not live at all well in captivity, especially in the unnatural confinement of the tubes. The decapod larvae showed their definite reaction to light by swimming constantly at the top of the tube and at the side nearest the oblique source of light. From this observation and their behaviour in jars and bowls while being picked out, there is no doubt that, had they been free to do so, they would have continued to swim along, horizontally near the surface and towards the source of light. It is possible that this overriding reaction towards light masked any effect on them of the phytoplankton, but it is also possible that they have no specific reaction under these circumstances.

No experiments have yet been performed expressly to search for the mechanism underlying this increase in upward migration in the presence of phytoplankton. The presence of plant cells must reduce the light intensity in the experimental tubes. The possibility of this being the direct stimulus is, however, rendered unlikely by the fact that the reduction in intensity in the *Chlorella* and Flagellate One experiments must have been as great, if not greater, than that in the rest of the series; and that the difference was still encountered in those experiments performed in the dark.

Experimental results such as those so far described must only be applied with the greatest diffidence to the problems found in nature itself. There are, however, certain extenuating circumstances in the present case. Foremost, in considering the problems of the inverse distribution of zooplankton and phytoplankton in the sea, is the difficulty of obtaining any other insight into the mechanisms involved. The most intensive sampling of plankton and the collection of physical data over a prolonged period of time from the same body of water (which may in the course of the months necessary for the study move hundreds of miles) is finally the only certain way in which the problem can be resolved. Such an investigation presents almost insuperable practical difficulties and certainly insuperable financial ones. Under these circumstances, and in view of the fact that such underwater observations as have been made confirm that the behaviour of the animals under experimental conditions is essentially like that in the sea, we may proceed to a projection of the experimental results into the field. During the process, however, the propriety of this operation must constantly be under review.

THE SIZE OF PLANKTON CONCENTRATIONS

Before discussing the consequences of the occurrence in the sea of such migrations as have been shown experimentally, it is necessary to consider both the sizes of phytoplankton and zooplankton patches and the density of cells in the former.

Phytoplankton

There is a great body of evidence, collected by naturalists and seafarers, concerning the size and shape of patches of discoloured water which have been seen from on board ship. Scoresby, as early as 1820, records long bands and streams of water apparently discoloured by diatoms; Darwin (1839) refers to bands and lanes discoloured by *Trichodesmium*, and Hornell (1908, 1917) and Allen (1921, 1928, 1938) refer especially to patches of red water caused by various flagellates. These observations are generally accompanied by estimates of the size of the areas, and maps may even be given, as in Suffren (1951).

The second method of delimiting areas of high phytoplankton density involves intensive sampling with nets, and is very laborious. Savage (1930), Savage & Hardy (1935), and Savage & Wimpenny (1936) record the results of intensive sampling in the North Sea and give charts on which the geographical distribution and size of diatom patches are depicted. Hardy & Gunther (1935) and Hart (1934) give the results of sampling work in the Antarctic and reveal similar sized concentrations.

Thirdly, the size of patches may be estimated from records taken with the Hardy continuous plankton recorder (Hardy, 1936; Lucas, 1940; Lucas & Macnae, 1941).

These references make it clear that the occurrence of patches of both diatoms and flagellates is a normal state in the sea and that these patches, as was so aptly stated by Scoresby, are 'of very variable dimensions'. The lowest size estimate is 20 or 30 yards across but much longer, and the highest 200 miles by something of the order of 40 miles; while a general mean might be considered 3 or 4 miles by half a mile to a mile. There is abundant evidence that, although some patches are diffuse, they may often have quite distinct boundaries and may extend to considerable depths. The most interesting feature is undoubtedly their shape—almost invariably long and narrow. Even where precise dimensions are not given words such as 'bands', 'streaks', 'lanes', and 'stripes' frequently occur. It could most reasonably be expected that any patches would be roughly circular in shape or at any rate irregular; the former might arise from some one point of origin that was particularly well seeded with the species, and the latter in a more diffuse manner from several centres. The possible action of currents or of wind in influencing these shapes must of course be considered. Tidal streams and movements in coastal localities frequently result in the collection of flotsam and jetsam into lines, but this is unlikely to happen in the open sea. Wind action may be thought too superficial, but it is known that it can produce lines of convergence and upwelling orientated parallel with its direction (Langmuir, 1938; Woodcock, 1944) and that the circulations causing these lines may extend as far as 20 m. down. Such an effect on a sufficiently large scale could influence the shape of patches but as so far demonstrated it appears to be too limited in extent. The possibility that grazing may be the cause will be considered later.

Zooplankton

Although the evidence is far more limited, reference to Hardy & Gunther (1935), Hardy (1936), Wimpenny (1936), and Rae & Fraser (1941) makes it clear that patches of zooplankton of the same order of size as those of phytoplankton are to be found in the sea. While recently, Barnes & Marshall (1951) have shown, by a statistical analysis of the results of a large number of small samples, that a number of small zooplankton organisms are often to be found in small swarms or clumps which are discontinuous in three dimensions. These patches are of a much smaller order than any previously demonstrated.

The Density of Phytoplankton Concentrations

Estimates of the density of cells in patches of diatoms can be found in Johnstone (1908), Gran (1912), and Allen (1919), who give, amongst others, values of 6·0, 0·5 and 0·46 cells/mm.^{3} for different organisms; while Harvey *et al.* (1935) give 45 cells/mm.^{3} for *Skeletonema* in Loch Striven. Flagellates and nanoplanktonic forms were not measured so exactly until later, when their great value as a source of food came to be realized. Gaarder & Spärck (1932), Alvik (1934), and Gaarder (1938) give values for Norwegian oyster polls and fjords, and Cole (1939) values for water pumped into oyster tanks at Conway. These are of the order of 4–24 cells/mm.^{3}. The highest values for natural water appear to be those of Gross (Marshall, 1947), who records 2400/mm.^{3} of flagellates for the water of Sailean More, the arm of Loch Sween used as a control in some fertilization experiments. The comparable figure for diatoms is 1·2/mm.^{3}.

Figures for more open waters as in Savage *et al.* (1935–6) and Mare (1940) are generally lower, but Cole & Knight-Jones (1949) give 5–70 organisms/mm.^{3} as general in the open sea. Estimates may be made from the larger hauls reported in Hardy & Gunther (1935) and Hart (1942); these give figures of 0·50 and 0·42 cell/mm.^{3} respectively of diatoms, but in one instance 25 cells/mm.^{3} of *Chaetoceros socialis* in Deception Island Harbour. Davis (1948) records 60 cells/mm.^{3} of *Gymnodinium brevis* during a red-water bloom in the Gulf of Mexico.

It is thus clear that the density of phytoplankton organisms in the sea can, on occasions, almost equal the densities attainable in enriched culture media, but that generally it is much lower than this. At present our interest is in customary and regularly attainable maxima rather than average concentrations over wide areas. The former may be taken to be of the order of 10 cells/mm.^{3} for diatoms and 100 cells/mm.^{3} for flagellates, although much higher figures than these may be found, as for instance 31·6 cells/mm.^{3} for diatoms and 2400/mm.^{3} for flagellates. Such concentrations must, however, be considered localized and of limited duration. More usual maxima would be 0·5 cells for diatoms and 50 cells/mm.^{3} for flagellates.

Those experiments performed at concentrations much higher than these are of especial interest in connexion with the exclusion hypothesis; as any excluding effect might be expected to be more marked at such concentrations. Those performed at concentrations more comparable with the ones found naturally are of especial interest in connexion with the new suggestion of feeding migrations.

CONCLUSIONS

Accepting then that patches of phytoplankton, of a comparable order of concentration to those in the tubes, occur commonly in the sea and that they are of the sizes indicated, what would be the consequences if the animals behave in the sea as they do in the various pieces of apparatus?

First it may be deduced that the hypothesis of animal exclusion cannot be of such universal application as was originally envisaged. The excluding effect was thought to be due to any concentration of phytoplankton (particularly diatoms) and to be of general and constant occurrence; affecting the vertical migrations of the great majority of animals in direct proportion to the concentration of plants. None of the diatoms used in the experiments has shown any toxic or excluding effect (with the exception of the highest concentration of *Nitzschia*). Such an excluding effect as has been shown is restricted to fairly high concentrations of two kinds of flagellates. This has, however, involved an active avoidance in the horizontal tubes, and what would amount in the sea to a passive avoidance, in the vertical tubes. It has been suggested by Hardy and by Lucas (personal communications) that exclusion might be mediated by only some specialized diatoms or flagellates. If that should be so and the responsible organisms should be of the sort found in the present work, then, in the mixed concentrations found in the sea, the excluding effect of the few would constantly be pulling in the opposite direction to the attractive effect of the majority of phytoplankton forms. There is insufficient evidence available on the distribution of flagellates in the sea for it to be stated firmly whether or not the widespread inverse relationships found could result from the wide occurrence of some particular toxic flagellate but this would seem to be a very unlikely mechanism. Indeed, Hart (1942) makes it clear that nanoplanktonic forms are not nearly so abundant in the Antarctic plankton as elsewhere and that the latter may often be almost purely diatom.

It would seem most consonant with the results to suggest that the exclusion mechanism as a means of producing the inverse relationship is of much more restricted occurrence. It could well be envisaged as operating where there are intense, often monospecific, blooms of some toxic flagellate, as for example *Goniaulax*. Under these conditions vertical migration up into the patch could easily be suppressed and there could also be an active horizontal migration away from the area by such animals as are equipped to do this.

This denudation of animals would allow unsuppressed division of the flagellate and the development of an even more repellent and larger toxic area. The marked avoidance by mysids of the concentrations of bacteria is of interest in this context. One of the effects of toxic blooms is to cause a concentration of dead fish and other animals in the area (Gunther, Williams, Davis & Walton Smith, 1948; Hornell, 1917). These decompose and must result in bacteria-rich water at least as concentrated as that used in the experiments. These regions will then be avoided by zooplankton forms, and this reaction should be classed as an exclusion effect.

Pure blooms of toxic organisms are however a rather specialized case, and such flowerings as do occur must frequently contain an admixture of attractive forms. Under these circumstances the reactions of animals must depend upon both the relative concentrations of the different forms and upon the physiological state of the animals themselves, and may be quite variable. Harvey (1937) has demonstrated what is apparently a selection in the feeding of *Calanus*. If this should occur in the sea, and acceptable forms should be taken in preference to harmful ones in such mixed patches, then the proportion of toxic organisms to others would be automatically and gradually increased until, perhaps, an almost monospecific bloom capable of excluding animals resulted. Such selective grazing could not account for blooms of organisms other than toxic or unaccepted forms.

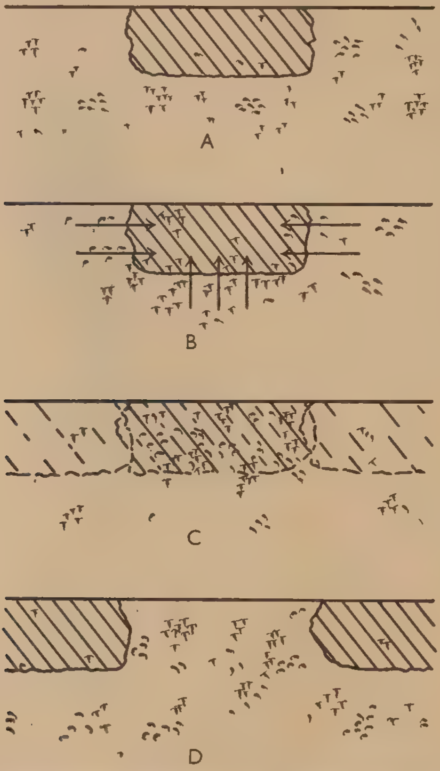
The most normal condition in temperate seas, however, must be the occurrence of patches of phytoplankton which, upon all the evidence available, will be both acceptable and attractive to the animals. If the principles of migration which have become apparent during the experimental work obtain also in the sea, then there must be a gradual accumulation of animals in areas rich in phytoplankton. This will be effected in two ways, that is by vertical and by horizontal migration.

Hardy has developed the conception of, as he terms it, 'planktonic navigation'. The basis of this hypothesis is a variable vertical migration. It is well known that the water layers at different depths travel at different speeds and a vertically migrating animal must, if altering its behaviour in different areas and depths of water, automatically alter its position relative to a fixed point on the surface. Animals can thus by swimming up more, or less, in particular areas collect in these or avoid them. This valuable idea is fully dealt with in Hardy & Gunther (1935, pp. 343–56). The principle does not require volition on the part of the animal and must, without any doubt, work continuously so long as the animals indulge in vertical migrations and so long as these alter according to the nature of the surrounding water. It is precisely such an alteration in behaviour which is demonstrated in the vertical experiments, but the alteration is directly opposite in sign to that originally postulated for the majority of animals by Hardy. The mechanism of navigation must still obtain, however, and must necessarily result in a gradual accumulation of animals in diatom-rich areas and a denudation of the neighbouring less rich areas. Indeed three species—*Calanus simillimus*, *Drepanopus pectinatus* and *Antarctomysis maxima* are cited by Hardy as behaving in this opposite manner and being attracted towards phytoplankton. These he regards, however, as interesting but special cases.

This migration into phytoplankton patches will proceed also in the horizontal plane. It is known that *Meganyctiphanes norvegica* is able to swim vertically upwards at about 128 m./hr. for periods of several hours (Hardy & Bainbridge, 1951) and to be capable of bursts of 271 m./hr. (unpublished). These figures represent respectively 1 mile in 13 hr. and in 6 hr., and if such speeds should be maintained horizontally in the sea they must necessarily have considerable effect on the spatial distribution of the animals concerned. Certainly no more effort, and probably less, would be involved in horizontal as compared with vertical swimming. Those animals then, already named, which are able to travel appreciable distances horizontally will gradually move up any gradients that may exist into the rich areas. It has been suggested that such a mechanism could take place only on a very small scale because of the lack of any orientating factor in the sea. If it should depend on random horizontal movements by the animals, then their area of search must of course be greatly reduced and the principle could not be of much importance relative to some of the large patches that we know to exist. If, however, there is some orientating factor which will draw the animals on constantly in one direction, then they will cover a much greater area, and their movements relative to large patches will become of importance. Such an orientating factor may well be found, in the northern and southern latitudes, but not in the equatorial ones, in the sun. Although daily traversing the sky from east to west there will always be a component in its light which is predominantly from either the north or the south depending upon the hemisphere. Such a horizontally directive component, even if very small, could in the otherwise uniform sea, impose a general trend in one particular direction on the aimless excursions of animals. This would greatly increase their exploratory powers and their chances of finding, and remaining in, areas rich in phytoplankton would consequently be increased. The strange little piece of swimming behaviour observed in those small copepods that came up to the surface and then moved along horizontally may easily be a representation on a small scale of the sort of behaviour we are envisaging. In any case, whether directive or random movement be operative, there are patches of phytoplankton of sizes conveniently within the reach of all zooplankton forms.

This gradual accumulation of animals in the rich areas will result in an increase in the intensity of grazing there. This will have little effect on the actual concentration of plant cells present until the rate of removal exceeds the rate of increment by cell division. When this happens the whole relationship will change rapidly. In the matter of a day or two, or even hours, depending upon the numbers of animals present, the plant cells will be grazed down to a negligible number.

In the meantime, the neighbouring areas of sea will, by the departure of the zooplankton, have acquired those conditions necessary for a fresh outburst of diatoms to take place. This will be more or less uninhibited and will quickly reach high concentrations. The situation will thus be reversed and the animals will again be inversely distributed in relation to the plants but a new cycle of migration, grazing and growth will immediately be initiated. This is shown diagrammatically in Text-fig. 4.



Text-fig. 4. Scheme of grazing and migration cycle. A, initial state with inverse relationship B, start of migration with some grazing; C, completion of migration and heavy grazing; D, reversal of initial state and return to inverse relationship. Oblique hatching represents concentrations of phytoplankton.

The speed with which this cycle of events will take place must depend on several variables including the densities of animals and plants, the speeds of migration, and the rate of division of the plants and of grazing by the animals. These are all factors to which definite values can be ascribed and it should eventually be possible to describe such a cycle mathematically. This is not however within the scope of the present work. The only necessary assumption is that the rates of migration must not be too high as compared with the rate of division of the phytoplankton, or otherwise a perfectly uniform sea would result and any increase in phytoplankton would be eaten down before developing noticeably. The small, when regarded absolutely, but relatively large difference in the proportions swimming up in the vertical tubes would seem in this connexion to be reasonable in extent; while an all or nothing reaction with a threshold value of a fairly high phytoplankton concentration would suffice as regards horizontal movements. Further, if there should be a lag in the movements of the animals then, starting from the slightest inequalities in distribution, these will oscillate with gradually increasing amplitude until the largest possible differences come into being.

The evidence at present available would seem to be more in accord with this hypothesis than with the others so far put forward. The inverse relationship in the sea is never absolute, often not clearly marked and sometimes completely reversed. This latter state, where high concentrations of both animals and plants occur in the same area, cannot be explained on the exclusion hypothesis, nor would it be expected on a simple theory of grazing. A dense concentration of animals and a dense concentration of plants are mutually incompatible entities. Their occurrence together must produce a situation of instability. The possibility of their having grown up together is surely much less likely than that of the animals having only recently come into the area, and being at that point in the cycle immediately prior to overcoming the rate of division of the plant cells. This state of distribution, the more common reverse one, and the intermediate ones can all be accounted for on a combination of migration and grazing, as can also the various experimental observations.

Hardy & Gunther (1935) found three species directly correlated with high concentrations of phytoplankton. Two of these (*Calanus simillimus* and *Drepanopus pectinatus*) were species which showed a most marked vertical migration, coming up to the surface before, and remaining there longer than, most other forms; and the third was a mysid (*Antarctomysis maxima*). It would now seem possible that this positive correlation occurred because these three forms are such active swimmers and have well-developed powers of navigation and migration. They can perhaps collect in regions of high phytoplankton density more readily than other forms and, their total numbers being insufficient to produce a marked grazing effect, they would therefore more often be found directly correlated with the plants than other forms.

Lucas's (1936) results, which might at first seem incompatible with the new hypothesis, can in fact be interpreted quite simply. He found a greater number of animals visible in the unshaded portion of half-shaded tubes when no diatoms were present than when the water was rich in diatoms. This he ascribes to the production by the diatoms, in sunlight, of some substance distasteful to the animals and the consequent avoidance by the latter of this part of the tube. An equally reasonable explanation is that the mysids, generally negatively phototropic in captivity (Tattersall & Tattersall, 1951), swim away from the light in the enriched tubes where they are well fed, while in the unenriched tubes they are continuously making excursions out of the shaded part in search of food and consequently more are counted here.

Fleming (1939) shows mathematically that any large increase in grazing associated with an increase in the number of grazers will reduce the diatom population very rapidly and that similarly the development of dense diatom populations can only take place when the grazing is less than the production of new cells. He regards such increases and decreases generally as due to reproduction and death of the animals but remarks that similar results would be obtained by 'the intrusion of an outside population'.

When currents in a particular region are sweeping on undeflected for large distances, the theoretical effect of navigation as postulated by Hardy is to form the animals into long straight lines (Hardy & Gunther, 1935, p. 351). This aggregation he takes to occur in regions of poor phytoplankton, but the argument can as well apply to the formation of long patches or belts either within, or more probably at first, along the edges of regions rich in phytoplankton. These concentrations of animals will imprint, by grazing, a similar banded pattern upon the phytoplankton; both eating out bands and leaving bands of water free for fresh outbursts. Such a system, distorted and broken occasionally by cross-winds, could account for the prevalence in oceanic areas of long narrow patches of phytoplankton which have been observed so repeatedly.

The essential feature of this migration hypothesis is the dynamic and fluctuating property which it attributes to distributions in the sea. Harvey's hypothesis of grazing pictures the whole process as rather rigid and automatic with concentrations of zooplankton growing and breeding in one body of sea and naturally precluding any outburst of diatoms there; while this will occur unrestrictedly in other areas. Hardy's hypothesis pictures a more or less rigid pattern of phytoplankton distribution imposing, by its exclusive powers, an inverse pattern upon the animals. The migration hypothesis, on the other hand, pictures a constant fluctuation and movement of both animals and plants relative to each other; the former constantly searching for the latter in an endless cycle of horizontal and vertical migrations, and the latter constantly flowering up when the pressure of grazing is eased. Such a picture, although less precise, would seem more likely to be true of living organisms in an environment so unrestrictive to movement as the sea.

The attribution to some of the commonly termed 'planktonic' animals, of considerable powers of horizontal migration need cause little concern. If a definition such as Bigelow's[1] is accepted, then it is clear that these animals should simply be referred to the already existing category of nekton.

The idea of a directive swimming by plankton animals is by no means new. No less an authority than Gurney says (1924, p. 39): 'It seems likely that the larvae of Decapods and Euphausids are not so much at the mercy of the currents as might be supposed. It is not very unusual to find swarms of the larvae of one species in different stages of development, which seems to indicate a power of keeping together from hatching onwards, or of collecting in a suitable locality'; and 'Some species seem to have decided preferences for certain regions, if one may judge from the behaviour of the British species of *Leander*. Quite early stages of the larvae are taken singly or in small numbers near Plymouth Sound, but later stages are exceedingly rare in in-shore waters, although the adults are abundant. But as the time for transformation approaches, the larvae seek the shore.... Larvae are no doubt frequently carried by currents out of their normal habitat, and Foxon has given the examples of *Porcellana macrocheles* and *Calappa marmorata* which are sometimes so carried by the Gulf Stream to the shores of New England, but I believe that this is exceptional, and that the larvae for the most part have power to control their movements.'

Russell (1927) speaks of encountering a swarm of *Corystes* zoeas which was plainly visible from the ship; while 6 weeks later a swarm of *Corystes* megalopas was encountered in the same locality, and he considers the possibility of it being the same one. The megalopas, interestingly enough, were restricted to an area rich in *Rhizosolenia*. Macdonald (1927), in an account of the food and habits of *Meganyctiphanes norvegica*, speaks of congregations of both this form and *Thysanoessa raschii* in the Cumbrae Deep, on occasions when there was much detritus in the water, and also when *Calanus finmarchicus* was abundant. The euphausids were feeding upon these two, and the clear implication is that they migrated to and remained swarming in this place only while the food was available. Macdonald says he is not suggesting that the vegetable detritus or *Calanus* attracted the *Meganyctiphanes* to this particular locality, but the possibility of this having occurred is more plausible than that of their concurrence being fortuitous. He reports Bigelow as giving an account of a similar swarming of *Meganyctiphanes* in the neighbourhood of a sardine factory from which refuse was being dumped.

Euphausids can, especially in the Antarctic, be completely herbivorous. The likelihood of their migrating into areas rich in diatoms is surely high, and a similar activity on the part of other vigorously swimming forms also plausible. This, combined with the preferential vertical migration of the remaining organisms into areas rich in food, must automatically result in such a cycle as we have postulated; and the continued occurrence of this would give just such a degree of inverse relationship to catches of animal and plant plankton as is, in fact, found in nature.

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[1] Bigelow (1924): 'By *plankton* we understand all such forms as float or swim freely in the water, but which, however active, are unable to carry out voluntary horizontal journeys of any extent, though certain of them perform considerable vertical migrations under the directive influence of sunlight or of some other physical stimulus.'

Summary

An apparatus in which it is possible to observe the horizontal migrations of zooplankton in gradients of phytoplankton is described, and an account given of its use in experiments involving various organisms. A preliminary search for toxic organisms is also described.

The experiments comprise a demonstration of a migration by various animals into concentrations of the diatoms *Skeletonema*, *Thalassiosira*, *Biddulphia*, *Nitzschia* and various mixed cultures and the flagellates *Chlamydomonas*, *Peridinium*, *Dicrateria*, K, and *Oxyrrhis*. Cultures of *Rhodomonas* and *Gymnodinium* II are found to produce the opposite effect, and no reaction towards *Lauderia*, *Coscinodiscus*, *Eucampia*, *Syracosphaera* and *Exuviaella* could be shown. Results with bacteria cultures and various inorganic gradients are also discussed.

A second apparatus in which it is possible to observe the vertical migrations of zooplankton in the presence and absence of phytoplankton is also described, and an account given of experiments in it involving various organisms.

The experiments comprise a demonstration that greater numbers of animals swim up in the presence of mixed phytoplankton cultures, *Coscinodiscus*, *Skeletonema*, *Ditylum*, *Chlamydomonas*, *Gymnodinium* I and II and *Oxyrrhis* than in unenriched water. Flagellate One has no effect and *Chlorella* possibly depresses the number swimming up.

Under-water observations on the swimming movements of some of the animals used are described.

The possible application of the observations towards resolving the problem of the inverse distribution of phytoplankton and zooplankton in the sea is discussed, and the hypothesis is proposed that this may be accounted for by a combination of migration and grazing. It is considered that plankton animals must migrate both horizontally and vertically into patches of phytoplankton and, when present in sufficient numbers, graze these down very quickly. In the meantime fresh growths of phytoplankton will have occurred in neighbouring areas of sea now devoid of animals and the inverse relationship will be re-established. This changing cycle of distribution is thought to be continuous.

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APPENDIX I. LIST OF PHYTOPLANKTON ORGANISMS USED

Class	Order	Family	Name or number	Size (in μ)	Remark
Chrysophyceae	Chrysomonadales	Prymnesiaceae	Flagellate 3	5–6	Gold, 3 flagella
			Flagellate 4	6–12	Gold, 3 flagella
			Flagellate 10	5–7	Gold, 3 flagella
			Flagellate 25	8 × 5–10 × 6	Deep gold, 3 flagella
			Flagellate K	3–6 diam.	Deep gold, 3 flagella
			Flagellate Lancelot II, Clone 8	5–8	3 flagella
			Flagellate L 4 (IV), Clone 3	5–8	3 flagella
			Flagellate L 4 (IV), Clone 6	5–8	3 flagella
			Flagellate Lancelot I, Clone 4	3–7	Gold
			Flagellate L 4 (13/4) 2 L, Clone 1	3–6	—
			Flagellate L 4 (4/5), Clone 3	5–8	—
		Chromulinaceae	*Chromulina pleiades*	3·5–6	(Flagellate E)
			Chromulina pusilla	1·5–2	—
		Ochromonadaceae	*Pavlova gyrans*	3 × 4–6 × 10	—
		Coccolithophoridaceae	*Coccolithus huxleyi*	5–7	Milky coloured
			Syracosphaera carterae	12–14	Brown
		Isochrysidaceae	*Isochrysis galbana*	5 × 2–6 × 4	(Flagellate I)
			Dicrateria inornata	3–5·5	—
		Cyrtophoraceae	*Pseudopedinella* I	6 × 6–10 × 10	Pale gold
		Chrysocapsaceae	*Phaeocystis pouchetii*	1–2 mm.	Golden brown. Colonies of various shapes
		Unplaced	Flagellate 1	3–5	Pale gold
			Flagellate 5	3–5	Gold
			Flagellate 22	3–4	Deep gold
			Flagellate J	6–8 long	Gold
			Flagellate A	4–6	Gold
			Flagellate Lancelot I, Clone 1	2–4	Gold
			Flagellate Lancelot I, Clone 5	3–5	Gold
			Flagellate Lancelot I, Clone on 19/5	4–6 m.	Mixed with *Pyramimonas grossii*
			Flagellate Lancelot II, Clone 10	2·5–4	—
			Flagellate L 4 (13/4) 2 L, Clone 6	3–6	Mixed with a cryptophycean red 5–8 μ
Cryptophyceae	Cryptomonadales	Nephroselmidaceae	Flagellate 6	4 × 2·5–8 × 4	Red and bean-shaped
			Flagellate 6*a*	Slightly larger than Flagellate 6 but may be same species	
			Hemiselmis rufescens	4–8·5 diam.	—
		Cryptomonadaceae	Flagellate 23	15–25 long	Red
			Flagellate 23*a*	Slightly smaller than Flagellate 23 but may be same species	
			Flagellate 14	5–8	Red
			Flagellate 16	6–8	Red. Tailed
			Flagellate 19	6–10	Red. Tailed
			Flagellate 20	12 × 6–20 × 9	Red
			Flagellate 21	15–25 long	Red
			Flagellate 29	15–20	Red
			Flagellate Lancelot No. 16 (250 ml.), Clone 3	15–20	Red
			Flagellate L 4 (13/4) 2 L, Clone 6	5–8	Red. Mixed with 3–6 μ chrysophycean
			Flagellate 'Gross's μ'	—	—
			Rhodomonas sp.	—	Red. Mixed with *O. marina* and *N. closterium*
		Unplaced	Flagellate L 4 (13/4) 2 L, Clone 5	5–8	Red
Desmokontae	Desmomonadales	Prorocentraceae	*Exuviaella* I	10–18	—
			Exuviaella baltica	9–15 long	—
			Prorocentrum micans	30–40	Golden brown
			Prorocentrum triestinum	8–14	Golden brown
		Desmomonadaceae (?)	Flagellate 12	10–15	Golden brown. Probably a primitive form

APPENDIX I (*continued*)

Class	Order	Family	Name or number	Size (in μ)	Remarks
Dinophyceae	Gymnodiniales	Pronoctilucaceae	*Oxyrrhis marina*	10–30	Colourless. Mixed with *Rhodomonas* sp. and *N. closterium*
		Gymnodiniaceae	*Gymnodinium* I	6–20	Brown
			Gymnodinium II	6–22	Brown
			Massartia rotundata	12–14	Brown
	Peridiniales	Peridiniaceae	*Peridinium trochoidium*	20–30	Reddish brown
Chlorophyceae	Volvocales	Chlamydomonadaceae	*Chlamydomonas* III	6 × 4–12 × 8	Green
			Platymonas apiculata	7–10	Green
		Polyblepharidaceae	*Pyramimonas* sp.	6–8	Green
	Chlorococcales	Chlorellaceae	*Chlorella stigmatophora*	4–6	Green
	Ulotrichales	Ulotrichaceae	*Stichococcus* I	3–5 × 2. Rods	Probably *S. cylindricus* Butcher
Bacillariophyceae	Centrales	Coscinodiscaceae	*Coscinodiscus concinnus*	150–450 diam.	All the diatom cultures appear brown to a greater or lesser extent
			Skeletonema costatum	7–16 diam.	
			Thalassiosira gravida	17–62 diam.	
			Thalassiosira nordenskiöldii	12–43 diam.	
			Lauderia borealis	34–38 diam.	
		Biddulphiaceae	*Biddulphia sinensis*	120–240 long	
			Ditylum brightwellii	25–60 diam.	
			Eucampia zoodiacus	25–73 broad	
		Chaetoceraceae	*Chaetoceros decipiens*	12–78 broad	
	Pennales	Fragilariaceae	*Licmophora lyngbyei*	50–75 long	
		Naviculaceae	Naviculoid	*c.* 100 long	
		Nitzschiaceae	*Nitzschia closterium*	20–90 long	
			Nitzschia closterium (forma *minutissima*)	25–35 long	Containing some of the 'tri-radiate' form
			Nitzschia seriata	100 × 6	

APPENDIX II. LIST OF ZOOPLANKTON ORGANISMS USED

- Crustacea
  - Branchiopoda
    - Anostraca
      - *Artemia salina.*
  - Copepoda
    - Eucopepoda
      - Calanoida
        - *Calanus finmarchicus*, *Eurytemora hirundoides*, etc.
      - Harpacticoida
        - *Tigriopus fulvus.*
  - Malacostraca
    - Mysidacea
      - *Hemimysis lamornae*, *Praunus neglectus*, *P. flexuosus*, *Neomysis integer*, *Mesopodopsis slabberi.*
    - Euphausiacea
      - *Thysanoessa inermis.*
    - Decapoda
      - Various larvae.

J. Mar. biol. Ass. U.K. (1953) 32, 449–476
Printed in Great Britain

OBSERVATIONS ON THE BREEDING AND SETTLEMENT OF *MYTILUS EDULIS* (L.) IN BRITISH WATERS

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(Text-figs. 1–8)

The literature on *Mytilus edulis* is particularly extensive. The most comprehensive account yet published is that of Field (1922), who gives excellent descriptions of the anatomy, physiology and embryology, as well as the bionomics, of this species. White (1939) adds little additional information upon the breeding and growth of *Mytilus*. Pelseneer (1935) gives an extensive bibliography covering both breeding and growth, whilst of the more recent work upon the breeding behaviour, the contributions of Battle (1932), Whedon (1936) and Young (1942) are most important. The occurrence of larvae in the plankton has been described in detail by Thorson (1946) and others; Visscher (1927), Orton (1933), Kändler (1926) and many others have given much information upon the spat-fall and settlement in several localities.

In spite of this wealth of published data there still remains considerable doubt over the time of onset and the duration of the breeding period of *M. edulis*, especially in relation to environmental factors. In particular, few recent investigations on *Mytilus* from beds in the sheltered and estuarine areas of the British coasts have been published, whilst the conclusions to be drawn from the older work (Herdman & Scott, 1895; Johnstone, 1898; Scott, 1900, etc.) are conflicting and lacking in precision. Finally, no attempt has previously been made to investigate the breeding, settlement and growth of this species simultaneously in a number of distinct geographical localities. This was the aim of the investigation, described below, which was commenced in 1946 as part of a general investigation of the breeding, settlement and growth of a number of common sessile littoral animals, and which is still in progress.

The author is indebted to Mr J. Baird of the Mussel Purification Station, Conway, to Mr J. Porteous of Portree, and to Mr R. Chestney of Brancaster for their invaluable assistance in supplying material regularly throughout the investigation. In particular, the author is indebted to the late Prof. J. H. Orton, F.R.S., for generously given advice and criticism, and to the Directors of Imperial Chemical Industries, Paints Division, under whose auspices the investigation was conducted.

Materials and Methods

The major part of the investigation was completed in 1946 and 1947. In the former year, samples of at least fifty specimens of adult *M. edulis* were obtained every two weeks from Liverpool (Prince's Landing Stage pontoons); Plymouth (Marine pier); the Blyth Estuary, Northumberland; Ramsey, Isle of Man, and also from one of the outdoor sea-water tanks at the Marine Biological Station, Port Erin, Isle of Man, where a supply of *Mytilus* taken originally from Ramsey was kept. In 1947, weekly (and sometimes more frequent) samples of at least 100 individuals were examined during the spring and early summer. Some of these were obtained from Liverpool, Port Erin and Ramsey as before, but the bulk of the material was obtained from Loch Portree, Isle of Skye; Conway, from the beds below the Benarth Road; and Brancaster Staithe (north Norfolk), from the Creek. Only one sample was received from Plymouth in 1947. Owing to the poor physiological condition of the *Mytilus* obtained from the Blyth Estuary during 1946, samples from this source were discontinued in the following year.

In subsequent years, the spawning of *Mytilus* was investigated at Brixham, South Devon, whilst the settlement and growth was followed closely at Liverpool in 1946 and 1947, and at Brixham in 1948, 1949 and 1950 under intertidal and continuously submerged conditions. Some observations on settlement were also made at Ramsey, Conway, Blyth, Portree, and Brancaster in 1946 and 1947, and at the River Dart in 1950.

Wherever possible, records of air and sea temperature were kept, and in a few localities salinity samples or records of salinities were also obtained.

The stages of gonad development were assessed from the colour and thickness of the mantle, and the degree of development of ova and sperm ascertained from the microscopic examination of smears. In some cases, sections of the mantle were prepared in order to establish the criteria of each of the five stages of development, described below.

Settlement of the spat and its subsequent growth were followed by observations upon the density and size of young *Mytilus* clustered upon the byssus threads of adults, or from the examination of non-toxic panels exposed for the purpose at Liverpool (1946–7) and Brixham (1948–50). Further information was obtained by the examination of submerged and intertidal substrata where and when opportunity arose.

Anatomy and Development of the Gonads

In *M. edulis* the sexes are separate, although in the early stages of development it is difficult to distinguish between them. In both sexes, the reproductive system consists of many branching ducts, which ramify throughout the mesosoma or 'abdomen' (see White, 1939, p. 33) and the mantle, and which, in a well-developed specimen, frequently spread into the connective tissue between the other internal organs, and over the digestive gland. In the mantle, the gonads extend as far as the pallial muscle.

Outgrowths from the ducts terminate in follicles, in which ova and sperm are produced. In males, the follicles are more uniform in size than those in the female. In both they are extremely numerous.

There are five principal gonoducts in the mantle and these converge on each side below the pericardium. From this point, the main genital canal, on either side, runs to the corresponding genital papilla, which is situated immediately anterior to the posterior adductor muscle. Field (1922, pp. 182 *et seq.*) gives a full description of the morphology and histology of the gonads.

Stages of Gonad Development

The development of the gonads was referred to five arbitrary, but readily distinguished, stages, including a 'resting-spent' stage in which no trace of sexuality is apparent, as follows:

Stage O. Neuter or resting spent stage. No follicles in mantle tissue.
Stage I. Onset of gametogenesis and appearance of follicles in the mantle.
Stage II. Follicles well developed and filled with unripe ova and sperm.
Stage III. Ova ripe and capable of fertilization, sperm activated by sea water.
Recently spent stage. Most of follicles empty; a few relict ova and sperm present only.

Stage O. The mantle characteristics vary according to the amount of reserve food material stored therein. When 'fat' the mantle is thick, yellow-cream or buff in colour, and of a very smooth appearance. It contains abundant glycogen and considerable fat. No ova or sperm can be seen and the genital ducts are usually obliterated by the great growth of connective tissue containing the reserve food products.

In starved specimens, or those in poor 'fattening' areas, where little accumulation of glycogen occurs, the neuter mantles are thin and semi-transparent, and in these the onset of gametogenesis is more easily distinguished.

Stage I. This stage includes all specimens showing the first signs of gametogenesis. In this stage, the ovarian and testicular follicles can be distinguished in the mantle tissue. Regeneration of the follicles commences at the outer face of the mantle, forming a single layer.

In the male follicles, only sperm mother cells and spermatids are present, whilst in the female, the oocytes are small and few have budded from the germinal epithelium.

In this early stage of the development, the colour of the mantle in each sex varies considerably, depending on the degree of development of the follicles and the amount and distribution of residual glycogen in the connective tissue. The female mantle is usually a red-brown or orange, and the male a light orange upon which background the small, but opaque follicles are readily apparent.

Stage II. In this stage well formed but unripe spermatozoa and ova are present in the follicles, which are well developed and prominent. Owing to wide variations in the nutritional condition of *Mytilus* from different localities, the thickness of the mantle (i.e. the number of layers of follicles) cannot be taken as a criterion of development. At this stage, however, the colours of the male and female mantles are distinct, owing to the mass of sperm or ova in the follicles. The male mantle has a brownish ground colour, which is almost obscured by the opaque white follicles. The female mantle is reddish orange or amber in colour, although the presence of ova bestows upon it a distinct apricot hue. Owing to the smaller size and more irregular distribution of the follicles, the female mantle appears to have a much smoother texture than the male mantle.

Stage III. Morphologically stage III is similar to stage II, except that the mass of the gonad is greater, and the connective tissue lying between the follicles in the mantle is even more occluded, although great variability occurs in material from the several localities, presumably owing to differences in nutrition. The general colour of the stage III female mantle is a very definite apricot, due entirely to the colour of the ova. The stage III male mantle is usually a full cream, or a yellowish cream in colour. The colour changes at maturity occur in individuals with mantles of any thickness. Thus within certain limits maturity appears to be independent of nutritional conditions, and ripe gametes can be obtained from poorly nourished individuals with thin mantles, whilst individuals with very thick mantle walls may yet contain unripe ova and sperm (see Young, 1945).

The ripeness of the ova and sperm in these mature individuals was determined from observations of their behaviour in sea water, and whether artificial fertilizations could be induced. The spermatozoa, when ripe, appear to be activated by sea water, whilst unripe sperm remains immotile. In no case has motile sperm been observed within the testicular follicles, and motility is induced solely by contact with sea water. Artificial fertilizations can be made with motile sperm only. Smears of the follicles of stage III males show few spermatids; thus it is clear that in *Mytilus*, as in *Pecten maximus* (Tang, 1941), a decline in spermatogenesis takes place as the gametes become mature. Occasionally individuals are found in which the gonoducts are filled with sperm; in smears of the follicles of these, no spermatids are found. The ripe sperm are arranged in lamellae converging towards the centre of the follicle.

In the stage III female, owing to the great development of the ovaries, the oocytes are of irregular shape. Within each oocyte is a large germinal vesicle containing a prominent nucleolus. The extreme growth of the follicles and the reduction in reserve food materials almost obliterates the interfollicular connective tissue. The ovarian egg possesses a distinct vitelline membrane about 1 μ in thickness, which readily bursts under slight pressure. The cytoplasm is very granular.

On being shed into sea water, the ripe oocytes rapidly assume a more or less spherical form (*c.* 0·068 mm. in diameter). During this process, maturation occurs, the germinal vesicle breaking down and the first polar body being budded off, the entire process occurring spontaneously in less than 20 min. at 12° C. Field (1922), however, states that fertilization is necessary before the first polar body can be budded off. Unripe oocytes do not round off in sea water, nor does the germinal vesicle break down.

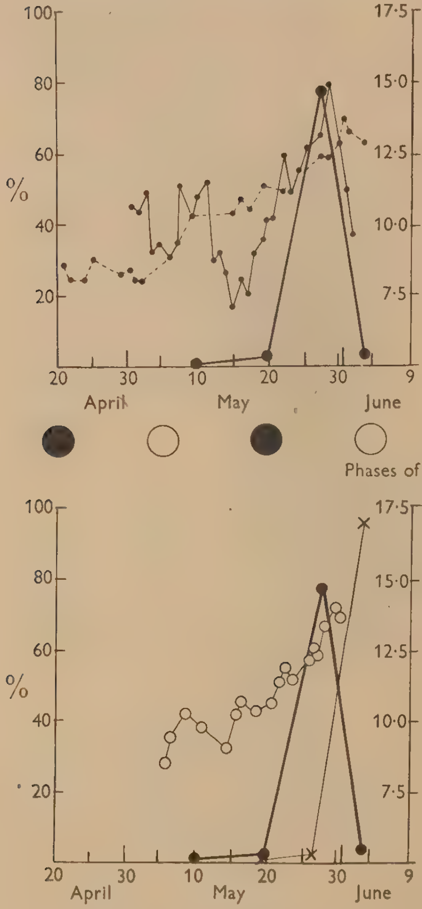
The breakdown of the germinal vesicle in sea water was regarded as a criterion of the physiological maturity of the oocyte, and was used as such throughout this investigation.

Recently spent. Recently spawned individuals of both sexes are readily distinguished by the semi-transparent mantle and mesosoma (see Field, 1922), usually of a reddish brown colour, in which a few relict ova or sperm can usually be found. It is only the presence of these residual gametes which enables the sex of the spawned individual to be determined. When the gametes are absorbed—probably by phagocytosis—all trace of sexuality is lost, and in *Mytilus* from most localities glycogen is rapidly accumulated in the connective tissue. It is unusual for less completely spawned individuals to be found, except at such times when spawning is slow.

The process of spawning has been observed *in vitro*. In both sexes the gametes are passed between the valves of the shell to the exterior, via the exhalant aperture. Sperm is emitted in a continuous stream, whilst ova are aggregated into short rods, which break up as they reach the exterior. No flapping movements of the shell have been observed. Emission of the eggs and sperm normally continues for 30–60 min., after which time spawning is virtually complete, only a few residual gametes remaining in the follicles.

SPAWNING BEHAVIOUR IN RELATION TO ENVIRONMENTAL CONDITIONS

Data obtained from samples examined during the spawning investigations carried out from 1946 to 1950 are given in Tables I–VII for Liverpool (1946, 1947), Plymouth (1946), Conway (1947), Portree (1947), Brancaster (1947), Ramsey (1947), and Brixham (1948, 1949), respectively. In order to assess clearly the state of sexual maturity in each sample, an 'index' or 'mean stage' of gonad development was calculated from the data, the number of individuals falling into each of the four developing stages (i.e. 'O' to 'III') being multiplied by a numerical factor equal to the arbitrary rating of the stage and the sum of these products divided by the number of individuals in the sample. Thus, a weighted mean stage of development is obtained, which has a minimum



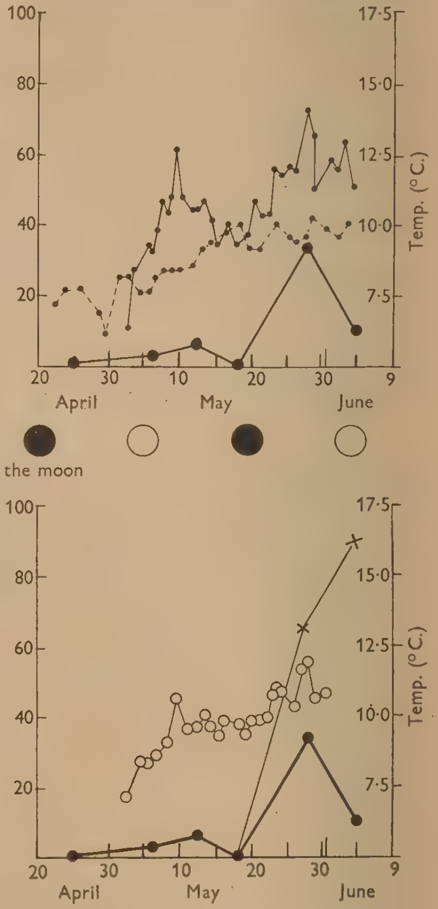


Fig. 1. Spawning of *M. edulis* at Conway in 1947, in relation to the lunar period and the sea and air temperatures, and the mean temperature to which the intertidal mussels were exposed during the spawning period.

Fig. 2. Spawning of *M. edulis* from the intertidal zone at Portree, Isle of Skye, in 1947.

●——●, % in sample recently spawned; ×——×, % resting spent; •- - -•, sea temperature (daily mean); •——•, air temperature (daily mean to which mussels exposed); ○——○, mean of sea and air temperatures.

value of zero, and a maximum value of 3·0 when the entire sample is sexually mature and ready to spawn. The weighted mean stage of gonad development for each sample is given in the tables.

The proportions of spawned males and females and of 'resting spent' individuals in each sample are plotted against time in Figs. 1–4 for Conway, Portree, Liverpool, Brancaster, together with the lunar phases, and daily sea temperatures. Where intertidal material was employed, mean air temperatures are also plotted.

TABLE I. BREEDING OF *MYTILUS EDULIS* AT LIVERPOOL

		% of sample									
		Female			Male						
Date of collection	No. in sample	Total	Ripe	Spawned	Total	Ripe	Spawned	Total % spawned	% Neuter	Total spawned and neuter	Index of gonad development
1946											
7. v.	75	48·0	25·3	1·3	49·3	26·7	2·7	4·0	2·7	6·7	2·39
17. v.	43	51·2	27·9	11·6	39·6	23·2	7·0	18·6	9·3	27·9	1·95
29. v.	50	52·0	6·0	46·0	22·0	4·0	18·0	64·0	26·0	90·0	0·30
13. vi.	45	2·2	0·0	2·2	4·5	0·0	4·5	6·7	93·3	100·0	0·0
27. vi.	31	0	0	0	0	0	0	0	100	100·0	0·0
16. vii.	43	0	0	0	2·3	0	0	0	97·7	97·7	0·023
28. viii.	29	0	0	0	0	0	0	0	100	100·0	0·0
16. ix.	33	16·1	0	0	21·4	0	0	0	63·6	63·6	0·36
22. x.	41	46·5	0	0	26·8	0	0	0	26·8	26·8	1·00
5. xi.	33	45·5	0	0	45·5	0	0	0	9·1	9·1	1·50
11. xii.	35	51·4	0	0	34·4	0	0	0	14·2	14·2	1·20
1947											
20. i.	19	36·8	0	0	63·2	0	0	0	0	0	1·31
27. ii.	25	40·0	0	0	48·0	0	0	0	12·0	12·0	1·08

TABLE II. BREEDING OF *MYTILUS EDULIS* AT PLYMOUTH

		% of sample									
		Female			Male						
Date of collection	No. in sample	Total	Ripe	Spawned	Total	Ripe	Spawned	Total % spawned	% Neuter	Total spawned and neuter	Index of gonad development
1946											
16. v.	42	57·0	42·8	4·7	43·0	43·0	0	4·7	0	4·7	2·76
3. vi.	45	48·9	11·1	37·8	51·1	8·8	42·3	80·1	0	80·1	0·60
17. vii.	49	22·4	0	8·2	10·1	0	4·0	12·2	67·5	79·7	0·36
29. vii.	58	8·6	0	1·7	6·9	0	3·4	5·1	84·5	89·6	0·19
13. viii.	69	23·2	0	5·0	11·6	0	2·2	7·2	65·2	72·4	0·46
28. x.	54	9·3	0	0	9·3	0	0	0	81·4	81·4	0·26
1947											
7. v.	69	45·0	1·5	0	43·5	1·5	0	0	11·6	11·6	1·25

From Fig. 4 it will be noted that, for a short period before spawning commenced, material from Brancaster was obtained from two sources, viz. the Creek, and a mussel pond, filled at high water, in which mussels are stored for marketing. The *Mytilus* obtained from this latter source were collected from the Creek and placed in the pond when the gonads were beginning to mature, two weeks before spawning took place. Daily temperatures of the water in this pond were taken, and from Fig. 4 it is clear that a considerably more rapid increase occurred here than in the Creek.

TABLE III. BREEDING OF *MYTILUS EDULIS* AT CONWAY

		% of sample									
		Female			Male						
Date of collection	No. in sample	Total	Ripe	Spawned	Total	Ripe	Spawned	Total % recently spawned	% Neuter	Total spawned and neuter	Index of gonad development
1946											
5. x.	99	36·3	0	0	51·5	0	0	0	12·1	12·1	0·95
23. xi.	102	52·8	0	0	46·1	0	0	0	1·2	1·2	1·16
10. xii.	48	45·9	0	0	43·8	0	0	0	10·3	10·3	1·83
1947											
27. i.	51	—	0	0	—	0	0	0	8·0	8·0	1·35
19. ii.	138	—	0	0	—	0	0	0	5·9	5·9	1·20
29. iii.	73	49·3	1·4	0	39·7	0	0	0	10·9	10·9	1·18
19. iv.	85	54·0	3·5	0	40·0	3·5	1·2	1·2	5·9	7·1	1·36
28. iv.	78	44·9	0	0	55·0	0	0	0	0	0	1·45
10. v.	107	51·4	5·6	0	48·6	1·9	0	0	0	0	1·59
20. v.	87	55·1	37·9	1·1	44·8	23·0	1·1	2·7	0	2·2	2·48
27. v.	84	41·7	0	33·3	55·9	0	46·4	79·9	2·4	82·1	0·35
3. vi.	110	0·9	0	0·9	1·8	0	1·8	3·6	97·2	99·9	0·0
16. vi.	97	0	0	0	0	0	0	0	100	100·0	0·0
1. vii.	165	0	0	0	0	0	0	0	100	100·0	0·0
15 vii.	83	0	0	0	0	0	0	0	100	100·0	0·0
28. vii.	57	—	0	0	—	0	0	0	91·5	91·5	0·9
14. viii.	40	12·5	0	0	7·5	0	0	0	80·0	80·0	0·20
24. ix.	57	26·3	0	0	22·8	0	0	0	51·0	51·0	0·65
1. x.	38	36·8	0	0	23·6	0	0	0	39·5	39·5	0·92
10 x.	50	38·0	0	0	48·0	0	0	0	14·0	14·0	1·13
31. x.	42	45·2	0	0	40·4	0	0	0	14·3	14·3	1·07
21. xi.	53	43·6	0	0	38·0	0	0	0	17·4	17·4	1·15

TABLE IV. BREEDING OF *MYTILUS EDULIS* AT PORTREE

		% of sample									
		Female			Male						
Date of collection	No. in sample	Total	Ripe	Spawned	Total	Ripe	Spawned	Total % recently spawned	% Neuter	Total spawned and neuter	Index of gonad development
1946											
16. ix.	32	9·4	0	0	6·6	0	0	0	84·0	84·0	0·16
22. x.	54	13·0	0	0	16·7	0	0	0	70·3	70·3	0·36
27. xi.	46	50·0	0	0	47·8	0	0	0	2·2	2·2	1·23
1947											
8. ii.	21	52·4	0	0	38·1	0	0	0	9·5	9·5	1·19
19. iv.	66	42·4	27·3	0	57·5	30·3	0	0	0	0	2·57
25. iv.	121	49·6	31·4	0	49·6	38·0	0	0	0·8	0·8	2·58
7. v.	85	43·5	37·6	1·2	54·0	41·2	1·2	2·4	2·4	4·8	2·69
12. v.	109	48·6	27·5	4·6	51·4	25·7	1·8	6·4	0	6·4	2·46
19. v.	177	39·6	31·7	0	60·4	44·6	0	0	0·5	0·5	2·76
28. v.	96	17·7	2·2	15·6	17·7	1·1	16·7	32·3	64·5	96·8	0·94
4. vi.	90	5·5	0	5·5	5·5	0	5·5	11·0	89·0	100·0	0·0
25. vi.	201	0	0	0	0	0	0	0	100	100·0	0·0

TABLE V. BREEDING OF *MYTILUS EDULIS* AT BRANCASTER

		% of sample									
		Female			Male						
Date of collection	No. in sample	Total	Ripe	Spawned	Total	Ripe	Spawned	Total % recently spawned	% Neuter	Total spawned and neuter	Index of gonad development
28. iv.	144	53·5	12·5	0	43·0	6·25	0	0	3·5	3·5	1·70
5. v.	157	42·0	14·7	1·9	58·0	21·6	2·5	4·4	0	4·4	2·17
10. v.*	131	45·0	27·5	3·8	55·0	25·2	1·6	5·4	0	5·4	
14. v.*	119	53·0	5·0	44·7	39·5	6·7	30·9	75·6	7·5	83·1	
14. v.	128	51·8	28·1	0	46·8	26·6	0	0	1·6	1·6	2·55
19. v.	133	54·1	25·6	3·0	41·4	18·1	2·3	5·3	4·5	9·8	2·24
26. v.	124	39·6	8·2	31·4	43·6	6·5	37·1	68·5	17·0	85·5	0·28
4. vi.	216	42·6	0	42·6	41·6	0	41·6	84·2	15·7	99·9	0·0
14. vi.	210	16·4	0·9	15·5	8·6	0	8·6	24·1	75·3	99·4	0·03
30. vi.	150	0	0	0	0	0	0	0	100	100·0	0·0
15. vii.	119	1·5	0	0·5	3·0	0	1·5	2·0	95·5	97·5	0·04
6. viii.	43	14·0	0	0	9·3	0	0	0	76·7	76·7	0·23
26. ix.	127	19·7	0	0·8	15·0	0	0	0·8	65·4	66·2	0·34
28. x.	46	41·4	0	0	43·4	0	0	0	15·2	15·2	1·00
19. xi.	37	59·4	0	0	35·1	0	0	0	5·4	5·4	1·24

* From Mussel Pond.

TABLE VI. BREEDING OF *MYTILUS EDULIS* AT RAMSEY (ISLE OF MAN)

		% of sample									
		Female			Male						
Date of collection	No. in sample	Total	Ripe	Spawned	Total	Ripe	Spawned	Total % recently spawned	% Neuter	Total spawned and neuter	Index of gonad development
1946											
18. vii.	40	15·0	0	15·0	12·5	0	12·5	17·5	72·5	100·0	0·0
30. viii.	49	0	0	0	0	0	0	0	100	100·0	0·0
12. ix.	100	14·0	0	0	7·0	0	0	0	79·0	79·0	0·26
6. xi.	48	6·2	0	0	20·8	0	0	0	73·0	—	0·33
2. xii.	40	15·0	0	0	15·0	0	0	0	70·0	—	0·53
1947											
3. v.	69	47·8	4·6	0	40·6	0	0	0	11·6	11·6	1·29
30. v.	48	20·8	0	20·8	12·5	0	12·5	33·3	66·6	99·9	0·0

TABLE VII. BREEDING OF *MYTILUS EDULIS* AT BRIXHAM

		% of sample									
		Female			Male						
Date	No. in sample	Total	Ripe	Spawned	Total	Ripe	Spawned	% Neuter	Total % spawned	% standard error of spawned individuals	Total % ripe
1948											
20. iv.	40	45	25	0	53	35	0	2	0	0	60
28. vi.	45	53	44	2	47	35	0	0	2	6·7	79
5. v.	58	47	35	4	50	41	5	3	7	3·3	76
12. v.	57	44	14	25	49	9	30	7	55	6·7	23
20. v.	58	36	9	22	41	5	29	22	51	6·6	14
1. vi.	42	14	2	10	17	2	10	69	20	6·2	4
16. vi.	40	25	0	0	5	0	0	93	0	0	0
1949											
7. iv.	25	44	28	0	56	44	0	0	0	0	84
14. iv.	51	47	39	0	53	41	0	0	0	0	80
21. iv.	36	56	39	3	44	30	3	0	6	4·0	69
29. iv.	75	43	27	11	52	33	16	5	26	5·1	50
3. v.	86	36	15	14	40	21	17	24	31	4·8	35
20. v.	41	29	2·5	22	37	5	24	34	46	7·8	7·5
25. v.	60	25	0	25	35	7	15	40	40	7·5	7·0
3. vi.	35	6	0	0	14	0	6	80	6	4·0	0

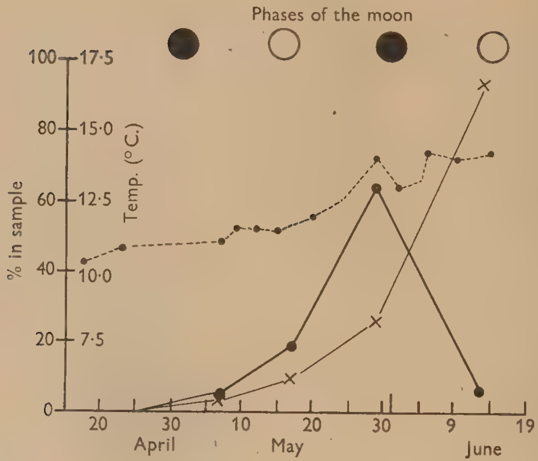


Fig. 3. Spawning of *M. edulis* at Liverpool in 1946. ●——●, % recently spawned; ×——×, % resting spent; •---•, sea temperature.

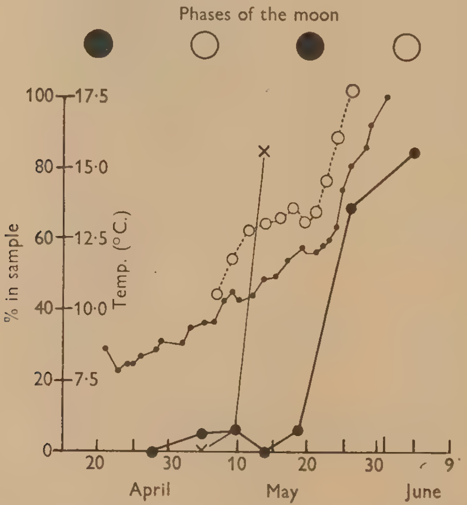


Fig. 4. Spawning of *M. edulis* at Brancaster Staithe, Norfolk, in 1947. ●——●, % recently spawned in creek; •——•, temperature in creek; ×——×, % recently spawned in pond; ○---○, temperature in pond.

The results for the material obtained from Blyth (1946) and for the material from the plaice tanks at Port Erin (1946) are not tabulated, as spawning had occurred before the first samples were obtained. In 1947 only two samples were obtained from Ramsey (Isle of Man), spawning having taken place

Table VIII. Summary of the Spawning Behaviour of *Mytilus edulis*

Locality	Date of commencement of spawning	Date of completion of spawning	Duration of spawning period (days)	Sea temp. range between spawning commencement dates (° C.)	Phase of moon at spawning and date	Approx. date of commencement of gonad ripening	Approx. temp. at commencement of ripening (° C.)
1946							
Liverpool	7. v.	13. vi	31	11·5–14·0	F.M. 17. v.	Mid April	8·5
Plymouth	16. v.–3. vi	3. vi	18	11·5–13·0	F.M. 17. v.		
Blyth Estuary	—	6. vi†					
Ramsay (I. of Man)	—	10. vi†					
1947							
Liverpool	19–27. v	6. vi	18	12·5–15·0	N.M. 20. v.	Early May	8·0
Ramsey	3–30. v	30. v	27	?	F.M. to N.M. 20. v.	Early May	
Brancaster (pond)	10–14. v	19. v	9	12·0–14·5	L.Q. 12. v.	Late April	8·0
Brancaster Creek	19–26. v	14. vi	26	12·0–15·5	N.M. 20. v.	Late April	8·0
Portree	19–28. v	4. vi	16	10·0–10·5 (9·5–12·0)*	N.M. 20. v.	Late March	6·5
Conway	10–14. v	19. v	9	11·5–13·0	N.M.	Late April	8·5
1948							
Brixham	5–12. v	1. vi	27		N.M.	March	9·0
1949							
Brixham	21–29. iv	3. vi	51	8·5–12·0	F.M. 13. iv. N.M. 28. iv.	March	6·5
1950							
Brixham	29. iv.–11. v	5. vi	37	10·5–13·0	F.M. 2. v.		
River Dart (Greenway)	28. iv.–18. v	18. v	21	10·5–13·5	F.M. 2. v.		

* Means of sea and air temperatures.

† Completed before this date.

between the dates of collection (see Table VI). Only small numbers of adult *Mytilus* could be found upon the pontoons of the floating landing stage at Liverpool in 1947, owing to severe storms during April, when the bulk of the population had been washed away. Observations were, however, made upon those which remained.

In Table VIII the spawning behaviour of *Mytilus* in the several localities is summarized, together with sea temperatures, etc.

In every case spawning commenced during the period late April–May and was completed within 3–4 weeks except at Brixham where a few (5%) ripe individuals were found a week or two later. Emission of ova and sperm during the first 7 days was always intensive, at least 70–80% of the population releasing its genital products (see Figs. 1–4). The remaining proportion of the population spawns during the ensuing fortnight or 3 weeks. No evidence of periodic spawning, nor of more than one discrete spawning period, was obtained in any locality during the 4 years of investigation.

Spawning, in each locality except Portree, commenced when the sea temperature had risen to 11·0–13·0° C. At Portree, however, the sea temperature (taken at high water) at the commencement of spawning was 10·0–10·5° C. If, however, the daily mean temperature to which the intertidal mussel bed was exposed during this period is computed from the maximum and minimum air temperatures, and the sea temperature, in relation to the times of high and low water, using a linear interpolation in estimating the air temperatures at the times of low water (where these do not correspond with the times of minimum and maximum air temperatures), it is shown that spawning commenced during a period when the daily mean temperature rose from 9·5 to 12·0° C. Similarly, spawning took place in the intertidal bed at Conway at the period when the daily mean temperature to which the bed was exposed rose from 10·5 to 13·0° C.

In the majority of the fourteen series of observations spawning was initiated during a period of spring tides, e.g. at Liverpool in 1946 and 1947, and Brixham in 1948 and 1949 after a new moon, and at Plymouth in 1946, and Brixham in 1949 and 1950, after a full moon. The mussels in the storage pond at Brancaster, however, spawned at a last quarter period, although those on the Creek beds commenced to spawn a week later, i.e. at new moon. Further, in each locality and in each year, spawning of *Mytilus* coincided with a period of predominantly bright sunny weather with little or no rainfall.

Period of Spawning

Of the many published accounts of the spawning period of *M. edulis*, two only refer to localities in which the present investigations were made, viz. Plymouth and the Isle of Man.

Matthews (1913) states that the spawning period of *M. edulis* at Plymouth in 1911 lasted from January until March, although samples which she took between May and August suggested that spawning took place in early spring. However, Matthews found that artificial fertilizations could be made only in late May, which suggests that natural spawning, in that year, took place at this period. The results of the investigations in 1946 (see Table II) are in conformity with this suggestion, for few ripe individuals were observed later than May. Additional evidence of a spring spawning at Plymouth is given by Lebour (1938) who states that the veliger of *Mytilus* is a principal constituent of the plankton in late spring and early summer.

Bruce (1926) obtained mussels from Ramsey, Isle of Man, and kept them for experimental purposes in a shallow concrete tank of running sea water at Port Erin. He states that spawning occurred at the end of May, eggs and sperm 'running freely on May 27th, 1925'. The results for Port Erin tank material in 1946 and for Ramsey material in 1947 point to a May spawning (see Table VI).

The majority of the published records of the spawning of *M. edulis* in British waters are based upon observations of the presence of larvae in the plankton or of settlement, although a few are based upon observations of gonad development. These records, in the main, suggest April and May as the spawning months (cf. Table VIII). In the Barrow and Morecambe Bay area, Herdman & Scott (1895) found mature *Mytilus* in May. Working in the same area and employing microscopic examination of the gonads as well as observations upon the plankton and upon settlement, Johnstone (1898) describes rapid emission of genital products in July 1898, but suggests that spawning may begin in April, and concludes that spawning occurs throughout May, June, July and August. Scott (1900) reports spawning of females only in tanks at Piel, and upon the Barrow beds, in May 1899, the emission of ova continuing until 13 June, when the males also spawned. In more recent years, Daniel (1921) gives April and May as the spawning period in 1921, but as late March in the following year (a warm year). The heavy spat-fall in Morecambe Bay in June 1933, reported by Orton (1933), suggests a May spawning (cf. spawning at Liverpool in 1946 and 1947).

McIntosh (1891) gives March–May as the spawning period of *Mytilus* in the Tees, Esk and Humber, whilst Williamson (1907) concludes that March and April are the months during which maturity occurs on the Scottish beds. At Millport, Elmhirst (1923) describes the spawning period of *Mytilus* as April to June, although Pyefinch (see Harris, 1946) finds that the heaviest settlement takes place during August and September. In a more recent paper (Pyefinch, 1950), however, he modifies this view extensively, suggesting that the heaviest settlement occurs in June–July. It would thus seem that spawning at Millport normally takes place in late May or early June.

There are a number of references to the spawning of *Mytilus* upon the western sea-board of Europe. Spärck (1920) states that spawning occurs in the Limfjord in May and June, and Thorson (1946) describes the sudden appearance of the veligers of *Mytilus* in late May or early June in the Øresund and the Isefjord. Kändler (1926), however, describes the occurrence of larvae of *Mytilus* at Heligoland over a very long period, although he states that a maximum occurs from April to June. Werner (1939), in reviewing the literature upon the spawning of North Sea lamellibranchs, suggests that *M. edulis* spawns from spring to autumn, and quotes in support the work of Havinga (1929) based upon the state of maturity of the gonads. On the other hand, Berner (1935), as the result of observations upon the gonads of *Mytilus* at Calais, states that the spawning period is February and March. The season of sexual maturity of *M. edulis* in the Gulf of Naples is stated by Lo Bianco (1899) to be March and April.

The spawning of *M. edulis* in the western hemisphere has been described by Stafford (1912), Field (1922), and Battle (1932). The former states that spawning takes place on the west coast of Canada in June, whilst Battle (1932) gives the spawning period as July to September in Passamoquoddy Bay. Field (1922) states that spawning commences on the Atlantic coast in April and continues until September. The work of Engle & Loosanoff (1944) suggests a principal spawning in late May in Milford Harbour, Long Island.

The past records of the period of spawning of *Mytilus* thus, in general, show considerable variation in the time of onset and duration, although a number agree with the observations described in this paper.

The Duration of the Spawning Period

In the present investigation the duration of the spawning period of *Mytilus* was followed on fourteen occasions. In eight of these, spawning was completed in 3–4 weeks (see Table VIII), in two in 9 days, whilst in two others (at Blyth and Ramsey in 1946) it was not possible to make an estimate of the duration of the spawning period. On the remaining occasions (at Brixham in 1949 and 1950) a few unspawned but ripe *Mytilus* (both males and females) were observed until 3 June in 1949, and 5 June in 1950, giving spawning periods of 51 and 37 days respectively. Observations at Brixham were too limited to enable the incidence of spawning to be followed during the spawning period in 1950, but in 1949 55% of the population had spawned during the first 14 days, whilst over 90% had completed spawning within 28 days from the commencement. It is possibly significant that, in both years, the sea temperature rose rapidly immediately prior to spawning, and then fell again, to rise slowly towards the maximum previously recorded. Further, the mean sea temperature at Liverpool in 1946 rose more slowly than in 1947, whilst the duration of spawning in the previous year was greater than in 1947 (31 days and 18 days respectively). The rapid spawning-out of the population at Conway and in the pond at Brancaster in 1947 may be associated with the rapid rise in temperature in those localities at the onset and throughout the period of spawning. It, therefore, seems possible that not only is the onset of spawning determined directly or indirectly by a rapid rise in temperature, but that the rate of increase of temperature subsequently influences the rate of spawning of *M. edulis*, a rapid increase of temperature producing a higher spawning intensity.

Once spawning was completed, in no locality or year was any evidence obtained of rapid re-development of the gonads, leading to a later spawning. The later maturation of the gonads of smaller and younger individuals, described by Jensen & Spärck (1934) was not observed in the present investigation.

Thus, in the localities investigated, spawning is limited to a period of 3–4 weeks in the late spring, during which time the entire adult population releases its genital products. This appears to be true even of semi-starved individuals with thin poorly developed mantles (e.g. those from Blyth).

Many writers in the past have ascribed a longer breeding season to *M. edulis* than the above. Care must be taken in comparing their findings with the present ones as many of the former are based upon the results of systematic plankton observations (e.g. Stafford, 1912, on the west coast of Canada; Kändler, 1926, at Heligoland; Borisiak, 1909, in the Black Sea; Thorson, 1946, in the Øresund; and Lebour, 1938, at Plymouth) or upon observations of intensity of settlement (Harris, 1946). In these instances there is no assurance of a homogeneous larval population (i.e. larvae spawned in one hydrographical area), and the long season of larval abundance or settlement may be due entirely to the incursion of larvae from other localities or from deeper beds in which the local hydrographical conditions cause a later, or earlier spawning. In this connexion, it should be noted that Thorson (1946) observed the presence of the larvae of *M. edulis* in the plankton over a considerably longer period in Øresund than in the relatively enclosed body of shallow water in the Isefjord.

Field (1922), although making observations in many localities on the eastern sea board of America, does not give details of the variation in the spawning period, and in America only Battle (1932) investigated the length of the breeding period of *Mytilus* by gonad examinations as well as plankton observations; she concluded that, in Passamoquoddy Bay, the spawning period lasts for 3 months.

Bruce (1926) and Daniel (1921) ascribe a short breeding period to *Mytilus*. The former states that spawning in laboratory tanks at Port Erin, which commenced in 1925 on 27 May, was completed by 5 June, whilst the latter observed a short spawning period on the Morecambe beds in 1921 and 1922 which were both warm years. He quotes Mr F. Gardner, the bailiff, as saying spawning occurs in April, the actual date varying according to the weather, and that some beds ripen before others. Berner (1935), too, finds that *M. edulis* at Calais spawns for a brief period only. Similarly, the fishermen at Brancaster Staithe, Norfolk, give the spawning period of the mussels in the creeks as April or May and state that the water becomes milky with milt suddenly, this condition lasting for a few days only, after which the mussels are thin and watery.

Thus, of those workers whose conclusions were based upon gonad examinations, only Battle (1932) and Havinga (1929, quoted by Werner, 1939), have found evidence of a lengthy period of spawning.

The intense initial spawning so strongly in evidence in most localities in 1947 has been noted by several workers, e.g. Field (1922), Daniel (1921), and Bruce (1926) and suggested by Thorson (1946), who observed a very sudden rapid increase in the number of larvae in the Limfjord, this high density lasting for a short time only.

It would thus appear that from observations upon the gonad condition, *M. edulis* may spawn during a period of 3–4 weeks, with the major release of ova and sperm occurring during the first 7 days, as was recorded in most localities in the present investigations, or that this species may spawn over a considerably longer period, the gonads possibly regenerating and discharging several times during the period (e.g. Battle, 1932; Havinga, 1929). At Plymouth and Brixham in 1946 and in 1949 and 1950 respectively the period during which a small number of ripe individuals could be found was greater than in other localities. Such observations suggest that where the initial rate of increase in temperature is small, the spawning period of *Mytilus* becomes protracted. In this respect it is significant that the rate of increase of sea temperature in the two localities in which Battle made her investigations (St Croix and Birch Cove, Passamoquoddy Bay) was low, the maximum temperature being slightly less than 14° C. in each instance, and the mean rate of increase 0·05° C./day during the spawning period. It appears probable that an upper limiting temperature to the regeneration of the gonads is achieved in those localities where spawning takes place during a short period, or that the temperature coefficient for laying down reserve materials is greater than that controlling the regeneration of the gonads; either or both would limit the spawning period in those localities where the sea temperature rises rapidly.

Ripening of the Gonads

In all localities, ripening of the ova and sperm commenced during a period of up to 6 weeks before the onset of spawning. This is shown clearly in Tables I–VII by the increase in the proportion of ripe individuals and the index of gonad development. Ripening of the gonads took place rapidly within 3 weeks of spawning at Conway and Brancaster in 1947, whilst at Portree in the same year a fair proportion of ripe individuals were found 5 weeks before the commencement of spawning (see Figs. 1, 2 and 4 for Conway, Portree and Brancaster respectively). Similarly, at Brixham in 1948 and 1949 some ripe individuals were present in samples taken during March, 5 or 6 weeks before the beginning of the spawning period. It is possibly significant that the minimum sea temperature at Brixham and Portree is considerably higher than at Conway and Brancaster (see Table IX), where the rate of increase in sea temperature from the annual minimum is greater, this greater rate of increase in temperature leading to a later but more rapid development of the gonads and a more rapid increase in the proportion of ripe individuals immediately before the occurrence of spawning.

TABLE IX. RATE OF INCREASE OF SEA TEMPERATURE

Locality	Seasonal minimum temperature (mean of 7 days)	Rate of increase in temperature from minimum to time of spawning
Portree (1947)	4·5° C.	0·066°/day
Conway (1947)	1·0° C.	0·151°/day
Brancaster (1947)	0° C.	0·142°/day
Brixham (1949)	7·5° C.	0·102°/day

A possible relation between sea temperature and rate of development of the gonads of *M. edulis* is further emphasized by the observation that the sea temperature at the commencement of the period of ripening is 7–8·5° C. in most localities where such estimates could be made (see Table VIII). It would thus seem that the gonads of *M. edulis* mature when the sea temperature is above *c.* 7·0° C., and that the rate of ripening is roughly proportional to the rate of increase in temperature.

There appears to be no direct relationship between nutrition and ripening of the gonads of *Mytilus*. Loosanoff (1942) has shown that *Mytilus* can ingest food at any time at temperatures above 0° C., whilst the observation that many *Mytilus* with very poorly developed mantles from Portree contained ripe ova and sperm, and spawned normally, suggests that maturity is not dependent upon good nutritional condition. Young (1942 and 1946) has also reached this conclusion in the case of *M. californianus*. In this connexion it is possibly significant that the glycogen content of *M. edulis*, as in *Echinus* and *Ostrea*, is reduced to a minimum value immediately prior to spawning (Daniel, 1921; Stott, 1931), suggesting that the development of the gonads occurs at the expense of reserve nutrient and is not dependent upon food ingested at this period, (see Fig. 8, p. 469).

During the period of ripening of the gonads in 1947, estimates were made of the proportion of mature ova obtained from the mantles of ripe individuals. As the spawning period approached, the proportion of ova rounding off in sea water, with the loss of the germinal vesicle, increased from a small value to over 90%. Artificial fertilizations made at these latter times were very successful, 80–90% of the fertilized ova segmenting normally. Further, as the gonads develop, an increasing proportion of the ova ripen at the same time in each individual.

Spawning Inducements

The rapidity of onset and the magnitude of the initial spawning phase of *Mytilus* strongly suggests that spawning is induced by a stimulus affecting the bulk of the population. That this stimulus is external is indicated by the variation in the length of the period during which a high proportion of mature individuals is found in the several localities before the onset of spawning.

The information derived from the present investigation strongly suggests that it is a rapid rise in the mean temperature to which the mussels are exposed to 11·5–13·0° C. which induces spawning (see Table VIII). It is also possible that the coincidence of the onset of spawning with a period of spring tides, in twelve of the fourteen instances summarized in Table VIII, is significant. Inshore temperatures, however, have been shown to rise in many localities during periods of spring tides in late spring and early summer, so that the apparent positive correlation between spawning and spring tides may in fact be due to the rise in sea temperature at new and full moon at this time of year.

Berner (1935) has described the spawning of *M. edulis* at Calais after a rapid change of sea temperature from 5 to 10° C., and a sudden decrease in salinity, whilst Nelson (1928) and Runnström (1929) give 10–12° C. and 14–16° C. respectively as the temperatures at which spawning takes place. Hutchins (1947) lends further support to the conclusion that *Mytilus* spawns at *c*. 11·5° C. in observing that the northern limit of distribution of the species is coincident with the 10° C. summer isotherm. Battle (1932), however, discovered no correlation between spawning and temperature in Passamaquoddy Bay. Whedon (1936), Coe & Fox (1942) and Young (1942, 1946) all state that there is no definite evidence that spawning in *M. californianus* is induced by temperature, or by temperature changes.

However, Battle (1932) found that the gonads of *M. edulis* at Passamaquoddy Bay matured at new moon during the summer months and that spawning followed immediately. Korringa (1947) implicitly suggests that the full moon spring tides were almost obliterated in this area in 1930, when the observations were made, and therefore ripening in normal years might occur at both new and full moon tides. Lunar periodicity in spawning is well known in many species (see Korringa, 1947, for an extensive bibliography), but the mechanism is extremely obscure. In *M. edulis* the effect of the greater variations in hydrostatic pressure at spring tides upon individuals with mature gonads may be of importance in inducing spawning. However, personal observations of spawning *in vitro*, and the observation of Bruce (1926) of rapid spawning in static tanks at Port Erin suggest that pressure is not a principal factor in stimulating mussels to spawn.

During 1947 attempts were made to induce *Mytilus* to spawn in the laboratory as the result of changes in environmental conditions. The results were extremely conflicting. It was found that mechanical shock (by pulling the byssus threads, or agitating vigorously) almost invariably produced a positive response from a number of the ripe mussels employed in the experiments. Young (1945) suggests that mechanical shock is the stimulus inducing spawning in *M. californianus* under natural conditions. Similarly, Field (1922) states that rough handling induces spawning in *M. edulis*, whilst Orton (1924) has shown that dredging operations during the breeding season can cause oysters to spawn. On the other hand, instances of spawning of *M. edulis* have been observed frequently under completely static conditions *in vitro*.

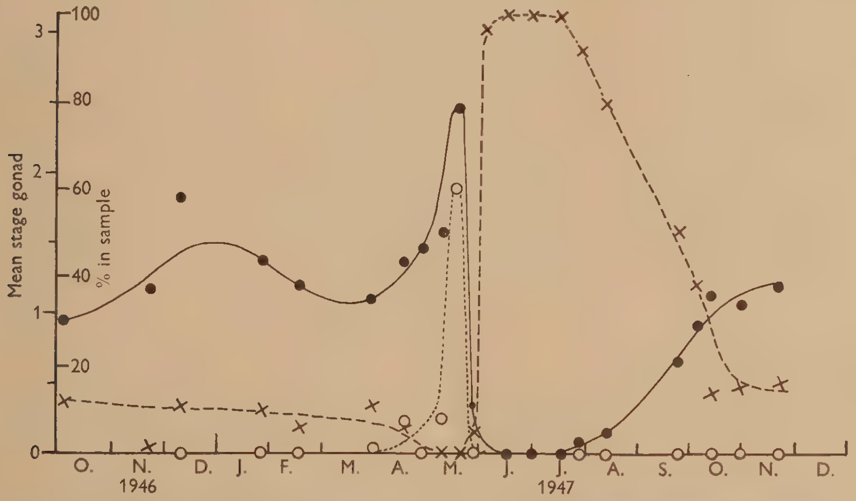


Fig. 5. *M. edulis*. Breeding at Conway, 1946–7. ●—— ●, mean stage of gonad development; × – – – ×, % of resting spent; ○······○, % of ripe adults.

Alternations of high and low temperatures, variations in salinity, and a mixture of mature sperm and ova with the sea water in which the experimental *Mytilus* were immersed produced conflicting results. Few experiments were made, however, and the results therefore are of little significance. A more detailed investigation of spawning inducements is now in progress.

Post-Spawning Changes in the Gonads

Within 10–20 days after spawning, a great regression of the gonads occurs. The decrease in volume on spawning is compensated to some degree by the intake of water (see Daniel, 1921), the mantle tissues becoming soft, spongy and semi-transparent. The residual ova and sperm are probably absorbed by phagocytosis by the cells of the follicle walls, and all trace of sexuality is lost. These individuals are in the 'neuter' or 'resting spent' stage. Growth of the connective tissue of the mantle and mesosoma also takes place immediately, and glycogen and some fat appear. During the latter part of June and throughout July, the amount of glycogen increases at a rate dependent upon the supply of food, until, by the end of July, *Mytilus* from good fattening beds (e.g. Brancaster and Conway), possess thick mantles filled with granules of glycogen. In general, there is no sign of the re-development of the gonads during this period, but at Plymouth in 1946 a small proportion of males and females could be distinguished in July, the proportion remaining constant until the final sample was taken in October 1946 (see Fig. 7).

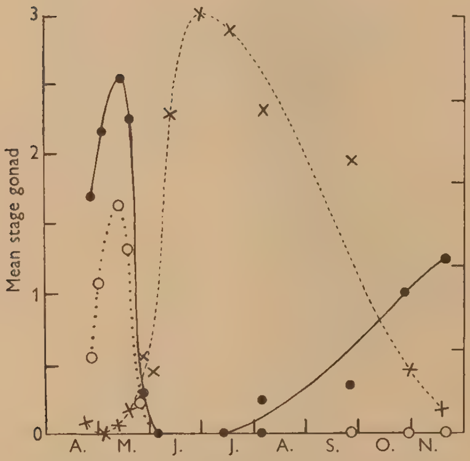


Fig. 6. The breeding of *M. edulis* at Brancaster Staithe, north Norfolk, in 1947.

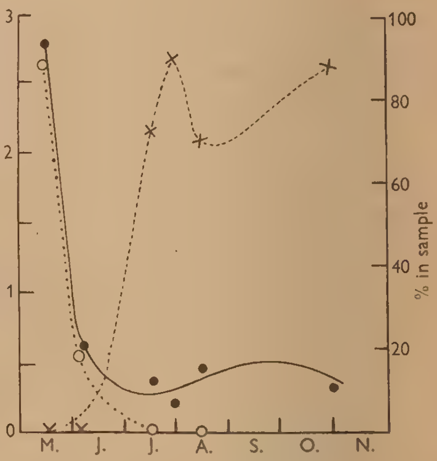


Fig. 7. The breeding of *M. edulis* at Plymouth, in 1946.

●—— ●, mean stage of gonad development; × –+– ×, % resting spent; ○······○, % ripe adults.

In the other localities the first traces of returning sexuality were observed in late July or during August, the gonads beginning to invade the mantle lobes in some specimens (stage I) (Figs. 5, 6 and 8). This process continues throughout September and October, until, by mid-November, the proportion of 'neuter' individuals is small. The connective tissue becomes concentrated as the follicles develop and the glycogen and fat become wedged between them (see Daniel, 1921).

From December 1946 until the end of March 1947, no further development of the gonads took place, but during late March and April a rapid increase in the mass of the gonads occurred, culminating in maturation and spawning in May.

Thus, in *M. edulis*, a non-reproductive period, during which large amounts of reserve food products are accumulated, occurs between successive reproductive phases.

The seasonal variations in the glycogen and fat content of *Mytilus* have been studied by Daniel (1921), who employed material from the Mersey and from Morecambe Bay. If Daniel's figures for the proportion of glycogen and fat per unit weight of the ash-free dry substance are compared with the index of gonad development for the Liverpool material employed in the present investigation (see Fig. 8) it is significant that whilst gonad development was stationary from December to March 1946, the glycogen content of *Mytilus* declined markedly, suggesting that some was used in the ordinary metabolic processes during a period of low temperatures and possible scarcity of food. This is also suggested by the continuance of the high value of the respiratory quotient after the period of rapid accumulation of glycogen (Bruce, 1926); the decrease in the R.Q. in March and April suggests the conversion of carbohydrates to fats, for storage in the maturing ova. It appears probable that scarcity of food, or the inhibition of assimilation of food by low temperatures, is responsible for the decrease in glycogen content during the early months of the year, as although Dodgson (1928) has reported the opening of the shells and the maintenance of ciliary currents in *Mytilus* even at 0·0° C., he further states that assimilation does not occur at temperatures below 4° C.

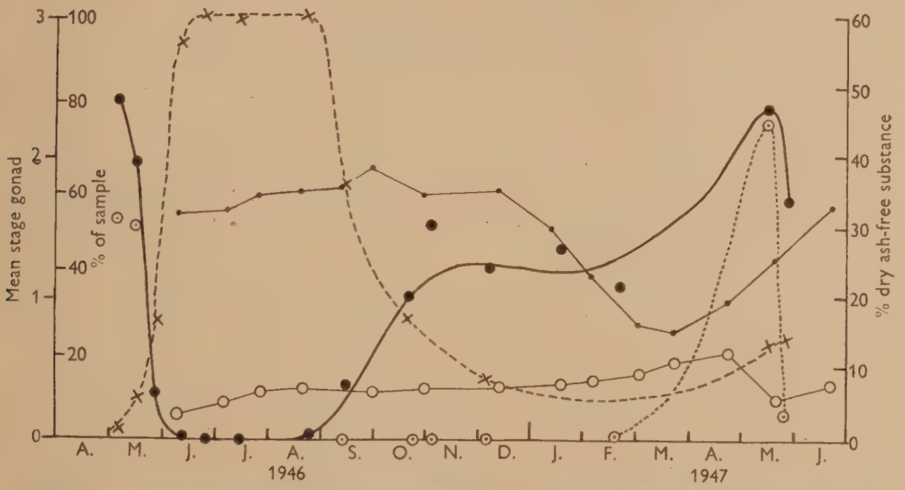


Fig. 8. *M. edulis*. Breeding at Liverpool, 1946–7. •——•, mean stage of gonad development. ×----×, % of resting spent. ●——●, % glycogen; ○——○, % fat; % composition by weight of ash-free substance, from Daniel (1921).

DEVELOPMENT OF THE LARVAE

Berner (1935) suggests that, in *M. edulis*, the sperm is shed into the sea and drawn into the mantle cavity of the female mussel, where fertilization takes place, the developing embryos being ejected when ciliated. No evidence to substantiate this has been obtained in the course of the present investigation; of the many mussels examined during the spawning period, none has been observed to contain developing larvae in the mantle cavity. Further, sperm from ripe males retains its activity in sea water at a temperature of 15° C. for at least 3 hr., and the ova, once discharged into sea water, are fertilizable for at least the same period.

Little exact information is available upon the length of larval life of *M. edulis*, or upon the size and age at which metamorphosis to the dissoconch stage takes place.

Field (1922) reared *Mytilus* larvae to the fifth day, after which abnormal development occurred. In the present investigation, a number of successful artificial fertilizations were made, the larvae remaining viable for periods of from 10 to 14 days in aerated sea water at a temperature of 16–18° C. After fertilization, the second polar body was budded off within 30 min., whilst the first division was observed to occur in from 1 to 2 hr. In 5 hr., many 8- to 32-celled stages were invariably present. Within 9 hr. from fertilization most larvae (pre-trochospheres) were completely ciliated and swimming strongly. In 24–30 hr., most larvae reached the characteristic trochosphere stage, and showed very strong positive phototropism. In most cases, the gut was first observed in $2\frac{1}{2}$–3 days after fertilization, whilst on the fourth day, many larvae possessed developing pro-dissoconchs. Fully developed veligers were usually present on the fifth to seventh days, the size being *c.* 100–110μ long and 80μ broad. Owing to the lack of suitable food organisms no further development occurred, although living larvae persisted for a further 3–7 days. No dissoconch larvae were observed, and no significant increase in size was recorded.

Estimates of the duration of larval life in the sea, based upon observations of the onset of spawning and the commencement of settlement, are accurate only if the larval stock is homogeneous, and if larvae developing from the initial spawning do not suffer a heavy mortality. Thus, the estimates based upon the observations set out in Table X cannot be taken as strictly accurate, but only as an indication of the probable duration of larval life. As a result of these observations, it would seem that the duration of the period between initial spawning and the commencement of settlement is 3–4 weeks. However, the normal larva life may well be 4 or 5 weeks, for in most of the localities in Table X the maximum rate of settlement was attained 2 weeks after the first few spat were observed. Battle (1932) records the occurrence of larvae 310–360μ in length in 14–21 days, whilst Field's (1922) figures combined with those of Matthews (1913), suggest a considerably lower rate of development, metamorphosis and settlement occurring in *c.* 10 weeks.

From the sizes of spat found upon panels exposed for periods of 7 days during the period of settlement of *M. edulis*, settlement can take place at any time or size after the dissoconch is formed, i.e. from a length of 400 μ to 1 mm. Thorson (1946) comments upon the great variation in size at which metamorphosis occurs (250–400 μ) and also the great differences in the rates of development, as shown by gill filament differentiation at different sizes.

Nelson (1928) records the ability of the advanced larvae to float near the surface by means of a gas bubble within the valves of the shell, even at a size of 900 μ. Attachment is effected initially by the foot, after the larva has become stereotropic. Nelson (1928) states that many surfaces may be explored before attachment by the byssus takes place. The foot is frequently employed as a means of locomotion on the surface film of the water.

SETTLEMENT OF THE LARVAE

In general, the period of settlement of *M. edulis* in British waters lasts from May to early July. The observed duration of settlement of the spat of this species in five localities (see Table X) is from 4 to 7 weeks. In each case it is probable that the extended period is largely due to the incursion of larvae from later-spawning beds in the same general locality. This is exemplified by the occurrence of two distinct maxima separated by about 2 weeks in the number of spat settling at Brixham in 1948. The long periods of settlement described by several workers (e.g. Harris, 1946; Pyefinch, 1950) are possibly due to such an incursion of larvae. Thus a longer period of settlement would be more likely to occur in a deep-water locality, than in a shallow-water locality in which a greater degree of homogeneity in spawning might be expected.

TABLE X. ESTIMATED DURATION OF LARVAL LIFE AND OF THE PERIOD OF SETTLEMENT OF *MYTILUS EDULIS*

Locality	Date of commencement of settlement	Estimated larval life (days)	Duration of period of settlement (weeks)
Liverpool	31. v. 46–6. vi. 46	18	6
	13. vi. 47–20. vi. 47	24	5
	8. vi. 48–15. vii. 48		6
Conway	3. vi. 47–16. vi. 47	21	4
Portree	4. vi. 47–25. vi. 47	21	6
Brancaster	4. vi. 47–14. vi. 47	25	5
Brixham	7. vi. 48–11. vi. 48	28	7
	29. v. 49–25. v. 49	26	6
	17. v. 50–31. v. 50	24	6

Mode (of 100) at 0·5 mm.

Plankton observations at Brixham in 1949 suggest that although *Mytilus* larvae may be present during July and early August in small numbers, settlement is rarely observed later than mid-July (see also Engle & Loosanoff, 1944).

Mytilus settles most abundantly in areas offering some degree of shelter from water currents and wave action. Settlement is invariably small upon clean smooth non-toxic exposure panels (e.g. glass, bakelite), but can be heavy on panels providing some degree of shelter by reason of the fouling already upon them. Settlement is always heavier upon inset panels than upon panels the surfaces of which are raised 0·5–1·0 cm. above the substratum; in this latter instance, settlement is greater in the depressions between panels, even where the panels are known to be completely non-toxic. Settlement is also greater upon the upper edges of these panels. The heaviest settlements always occur upon hydroid colonies, the rough or frayed surfaces of ropes, and rough shells. In raft exposures, *Mytilus* settles more abundantly in the darker positions, possibly owing to the colonization of the lighter positions by algae, with the consequent reduction in area upon which the spat can attach and grow. Initial settlement can, however, be quite high upon filamentous algae, but the mussels rarely persist, even in small numbers.

When settlement occurs upon a non-toxic panel freely exposed in the sea at a constant depth, it is invariably heavier at the upper edge, becoming rapidly smaller as the distance from the upper edge of the panel increases. In years of good spat-fall an entire panel may be fouled by *Mytilus*, but initially the upper edge and the surfaces bordering it bear more and larger mussels. As settlement declines, greater growth is usually observed where the density of settlement is less. A projection across the centre of the panel will give rise to a heavier settlement of *Mytilus* immediately below it than immediately above. It would thus appear that *Mytilus* spat accumulates where the surface upon which it settles is interrupted by some discontinuity. It is probable that the spat, after initial settlement, migrates vertically upwards upon the surface by means of its ciliated foot until a discontinuity is reached, whereupon it fixes itself by means of its byssus, the discontinuity providing a stimulus for byssus formation. A similar tendency to accumulate at the upper parts of panels, possibly for a similar reason, has been noted in the settlement of *Balanus crenatus* and *B. improvisus* at Liverpool and Burnham-on-Crouch (Chipperfield, 1948).

The spat of *Mytilus edulis* attaches to floating structures most abundantly from just below the surface to a depth of about 2 ft.; below this the density of settlement declines. This is clearly shown by counts of the number of mussels settled on 3 in. long sections of tubular steel panel holders $1\frac{1}{4}$ in. diameter, suspended from a raft at Brixham in 1948 and 1949, made towards the end of the period of settlement. The counts are set out in Table XI. It is apparent that settlement will also occur in the region of the nominal water line, and in that zone above the water line which is subject to constant immersion by wave-action, although the settlement falls off very rapidly in this region.

A similar distribution of settlement is seen in the case of fixed vertical substrata. In 1949 a chain covered with mussels and hanging freely to the bottom from a naval dolphin in Brixham Harbour, was denuded in parts for spawning investigations. Subsequently, the patches cleared of mussels were extended, and more made at known distances below approximate L.W.S.T. mark. Rough counts of the recently settled and surviving *Mytilus* in these areas were made on 14 July (see Table XII). Below L.W.S.T., settlement was not significantly greater at a depth of 15 ft. than at L.W.S.T. Above L.W.S.T., settlement was considerably smaller, no surviving settlement at all persisting at M.T.L. Similarly, at Liverpool in 1946, although a heavy surviving settlement occurred upon the low-tide floor of the jetty at the Prince's landing stage (see Corlett, 1948), that upon the mid-tide floor and upon the beams immediately beneath was extremely sparse.

TABLE XI. SETTLEMENT OF *MYTILUS EDULIS* IN RELATION TO DEPTH BELOW WATER LINE

Distance from water line (in.)	Numbers/3 in. length of 1¼ in. diam. vertical rod						
	1. vii. 48			16. vi. 49			
	1	2	Mean	1	2	3	Mean
+6 to +3	0	0	0	5	22	7	11
+3 to 0·0	8	15	11	35	28	58	40
0·0 to 3	33	43	38	101	85	158	115
−6 to −9	284	265	274	412	358	392	387
−12 to −15	221	294	257	329	400	360	363
−24 to −27	236	160	188	266	206	252	241
	Mean size: 4·5 mm.			Mean size: 3·0 mm.			

TABLE XII. INTERTIDAL SETTLEMENT OF *MYTILUS EDULIS* ON CHAIN, BRIXHAM, 14 JULY 1949

(Numbers (approx.) per link of chain. Mean size: 8·0 mm.)

Approx. level	No.	Approx. level	No.
H.W.N.T.	0	L.W.S.T.	50
M.T.L.	0	5 ft. below L.W.S.T.	180
M.T.L.–L.W.N.T.	0	15 ft. below L.W.S.T.	160
L.W.N.T.	8		

Engle & Loosanoff (1944) explain similar results by suggesting that settlement of *M. edulis* larvae occurs only at low-water slack, or at late ebb. It is possible, however, that settlement by adhesion to the substratum by the foot may occur at any state of the tide, but that the time required for secure attachment by the byssus to be completed is of the order of 4½–6 hr. As the byssus is secreted only when the mussel is immersed, spat adhering in positions higher in the intertidal zone would not persist unless they happen to be in a position in which they are constantly wetted, e.g. a small depression in a rock surface. Tidal currents and wave action are obviously complicating factors, but the restricted settlement of mussel spat between L.W.N.T. and M.T.L. on a rocky shore subject to some wave action such as Shoalstone Beach, Brixham, in narrow cracks and small hollows lends support to the suggestion detailed above. The effect of exposure to the air upon the physical and chemical properties of the liquid, or partly solidified, byssus material cannot be ignored in this context.

SUMMARY

During 1946 and 1947, regular samples of *Mytilus edulis* from a number of localities on the British coasts, including Conway, Brancaster and Liverpool, were examined for gonad condition and spawning. For each sample, the mean stage of gonad development was computed. The criteria employed in distinguishing the stages of gonad development are described.

Ripening of the gonads takes place within a few weeks of the onset of spawning, in general commencing when the sea temperature has risen above 7·0° C. There appears to be no correlation between nutritional condition and ripening of the gonads, or subsequent spawning.

In all localities and in each year in which observations were made spawning occurred in late spring (mid-April to the end of May) and in most areas lasted for a short period only (2–4 weeks). At Brixham, in 1949 and 1950, the duration of the spawning period was longer (4–6 weeks). In most cases, 70–80% of the mature population spawned during the first 7–10 days of the breeding period. No evidence of periodic spawning was obtained.

In all cases, spawning commenced in a period during which the mean temperature to which the mussels were exposed was rising from *c.* 9·5° C. to 11–12·5° C. In most cases, the onset of spawning was coincident with a period of spring tides, and of predominantly bright sunny weather. The initial rate of spawning appears to be directly related to the rate of increase in mean temperature to which the mussels are exposed.

After spawning, mussels enter into a 'neuter' or 'resting spent' stage in which all traces of sexuality are lost. In good 'fattening' areas, the connective tissue of the mantle and 'mesosoma' rapidly becomes packed with glycogen and some fat. Re-development of the gonads usually commences in July or August, 2–3 months after spawning. A rapid increase in the mass of the gonads occurs during early spring.

The period of free swimming larval life is variable and is probably usually *c.* 4 weeks. Settlement can occur at any time after the formation of the dissoconch, corresponding to a length of from 0·4 to 1·0 mm.

Settlement is heaviest on floating structures in the region between the water line and a depth of 2 ft. Below this level the density of settlement declines somewhat. Similarly, upon intertidal structures and upon beds in

estuarine areas, little persistent settlement occurs above M.T.L. and the heaviest settlements take place at and below L.W.S.T. Settlement almost invariably occurs initially at the upper edge of submerged panels, etc.

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J. Mar. biol. Ass. U.K. (1953) 32, 477–490
Printed in Great Britain

THE LARVAE OF THE SPATANGIDAE

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(Text-figs. 1–5)

Introduction

Mortensen (1927) has given an account of the distribution of the six species of Spatangidae which occur in British waters. Of these, four may be regarded as widespread and, in places, numerous: *Echinocardium cordatum* (Pennant), *E. flavescens* (O. Fr. Müller), *Spatangus purpureus* O. Fr. Müller, and *Brissopsis lyrifera* (Forbes). The fifth species, *Echinocardium pennatifidum* Norman, has been found on the south, west and north British coasts, and down to Durham on the east coast, though nowhere in large numbers. The sixth species, *Spatangus raschi* Lovén, is much more restricted, and is known only from fairly deep water off the west coast of Ireland and off the Shetland Islands.

Our knowledge of the larval forms of these species is not satisfactory. The larva of *Echinocardium cordatum* has been described by Mortensen (1898) and MacBride (1914). However, Thorson (1946) refers to the possibility that the larva of *E. flavescens* may be very similar to that of *E. cordatum*.

Chadwick (1914) described some young larvae which he assigned to *E. flavescens*, but Mortensen (1920) would not accept the certainty of this identification and gave *Brissopsis lyrifera* as a possible alternative. Despite the fact that Chadwick's drawings are poor (Mortensen, 1920, who saw the original material, pointed out that the post-oral arms are fenestrated, not simple as shown in the drawings), there is little doubt that Chadwick's identification was correct. It is a curious fact that Mortensen acknowledged seeing Chadwick's '*E. flavescens*' and gave *Brissopsis* as an alternative in the same paper (1920) in which he described the larva of *B. lyrifera*. As will be seen below, the larvae of the two species are, in fact, easily distinguishable even in very young stages. It has been accepted up to now that the larvae of *Echinocardium flavescens* are unknown (Mortensen, 1927; Thorson, 1946).

Young larvae described by Krohn (1853) were assigned by him to *Spatangus purpureus*. In this case, also, Mortensen (1913) questioned the identity. Mortensen (1913) has described the fully grown larva of *S. purpureus*, giving as its main characteristic the exceedingly long posterior process, but there is reason to believe (see below) that identification based on such a characteristic is unsatisfactory. The best account of *S. purpureus* larvae is given by Ohshima

Echinocardium cordatum. The postoral, postero-dorsal, and posterior arms (Fig. 1) are fully fenestrated, unlike those of the other three species. The 'tail' of the body rod is relatively long, a half or more the length of the main body rod. The earliest development of the calcareous plates is off the anterior connexion. The ventral horizontal rod is situated some distance posterior to the base of the postoral arm (and the anterior transverse rod). In the last

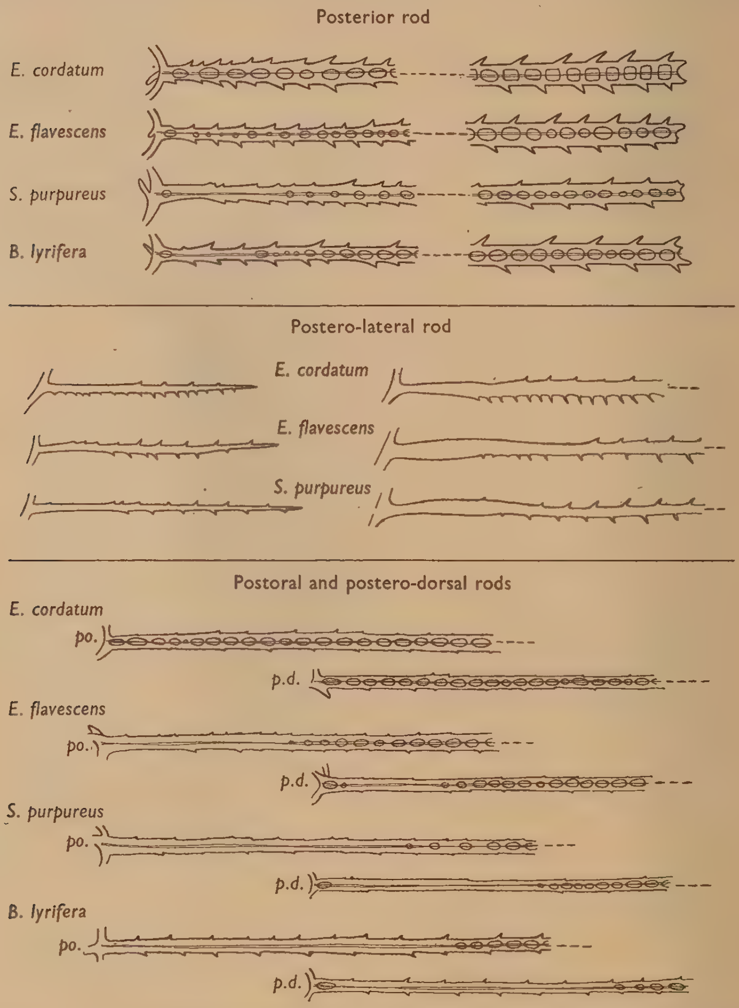
Posterior rod
E. cordatum
E. flavescens
S. purpureus
B. lyrifera
Postero-lateral rod
E. cordatum
E. flavescens
S. purpureus
Postoral and postero-dorsal rods
E. cordatum
po.
p.d.
E. flavescens
po.
p.d.
S. purpureus
po.
p.d.
B. lyrifera
po.
p.d.

Fig. 4. Typical examples of the posterior, postero-lateral, postoral and postero-dorsal arm rods of four species. Abbreviations: *po.*, postoral; *p.d.*, postero-dorsal.

stage, the support for the posterior edge of the calcareous plate of the postoral arm is markedly anterior to the place of origin of the ventral horizontal rod. The postero-lateral arm is very spiny; there are approximately twice as many spines on the posterior edge as on the anterior.

Echinocardium flavescens. The posterior arm is, with rare exceptions, fully fenestrated. The fenestrations in the proximal part may be fairly large or very small; rarely a section may appear to be unfenestrated. The proximal part of the postoral arm is unfenestrated to a variable degree (Fig. 5), the great majority (forty-seven out of fifty measurements) to a length of 0·10–0·17 mm. inclusive. The proximal part of the postero-dorsal is unfenestrated, apart from a single basal fenestration. Along the unfenestrated part there may occasionally be an isolated fenestration. The length of the unfenestrated part, from the base of the arm to the beginning of the regular series of fenestrations, is variable (Fig. 5), twenty measurements falling into the range 0–0·13 mm.

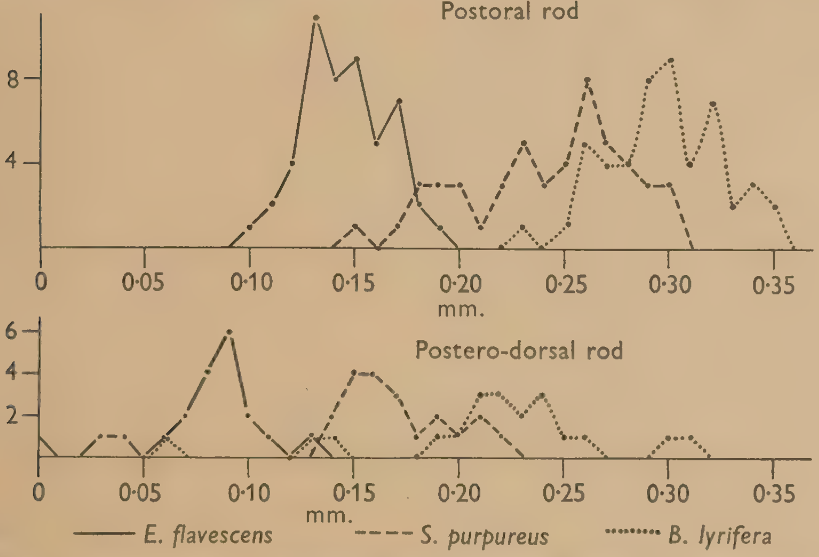

Fig. 5. Length-frequency distribution of the unfenestrated portion of the postoral arm (fifty measurements in each species) and the postero-dorsal arm (twenty measurements in each species) of *Echinocardium flavescens*, *Spatangus purpureus* and *Brissopsis lyrifera*.

The 'tail' of the body rod, relative to the full length of the body rod, is as long as that of *E. cordatum*. The earliest development of the calcareous plates is off the anterior connexion. The ventral horizontal rod is situated nearer to the base of the postoral arm than in *E. cordatum*, and the support for the posterior edge of the postoral calcareous plate is more or less at the same level as the ventral horizontal rod, or very slightly anterior. The postero-lateral arm

When present it is convenient to recognize a 'tail' in the body rod, that part posterior to the posterior connexion.

The late larva is very much larger and the rigid box of the young larva becomes transformed to cope with the increase in size. The skeleton is still box-like, but the anterior and posterior connexions have disappeared and the recurrent rod is replaced by the dorsal body rod which is part of the root of the fenestrated postero-dorsal arm. The anterior transverse rods are replaced by the ventral horizontal rods, which curve outwards to embrace the soft parts. Corresponding dorsal horizontal rods arise from the dorsal body rods. At each side are three calcareous plates, not shown in the diagram, which overlay and overlap each other. On the outside is a plate attached to the postoral arm and ventral body rod, on the inside a plate attached to the antero-lateral arm, and in the middle a plate attached to the root of the postero-dorsal arm. In some species (*Spatangus*) the tips of the posterior transverse rod become fan-like and overlay the plate complex on the outside.

Diagram C in Fig. 1 shows, in an intermediate stage, how the dorsal body rod, arising from the root of the postero-dorsal arm, lies parallel and outside the still existing recurrent rod.

The changes during development of the various parts of the skeleton are shown diagrammatically in Fig. 2. It should be realized that the structure is three-dimensional, and the diagrams in Fig. 1 should be kept in mind to aid an appreciation of the form of the larva. For convenience five stages are taken, but it must be emphasized that the development is gradual, and the choice of five stages is entirely one of expediency. The stages are shown in the left vertical series (A) in Fig. 2. In stage 1 are included larvae which have only two pairs of arms and the unpaired posterior arm (as in Fig. 1), and larvae in which the development of the third pair of arms, the postero-dorsal, has commenced. Stages 2–5 are shown in the diagrams of Fig. 2 A only by the dorsal arch and its associated arms. The disposition of the dorsal arch in the complete larva is illustrated in Fig. 1 B. In stage 2 larvae the dorsal arch and the beginnings of the pre-oral arms are present. In stage 3 larvae there are the rudiments of the antero-dorsal arms. In stage 4 the antero-dorsal arms are half-developed and stage 5 is the final stage.

The root of the postero-dorsal arm in stage 1 is a three-rayed star (Fig. 2B). By stage 2 one of the star rays has increased in size and become the dorsal body rod with a short branch, the dorsal horizontal rod. In later stages the dorsal horizontal branch increases greatly in size. The remaining two rays of the star elongate slightly, curve and coalesce to form the circumference of a calcareous plate which is developed by stage 5.

The posterior transverse rod bearing the posterior arm is three-rayed in stage 1 (Fig. 2C). Two rays elongate by stage 2 and by stage 3 the elongated rays have the rudiments of the postero-lateral arms, which increase greatly through stage 4 to their full length in stage 5.

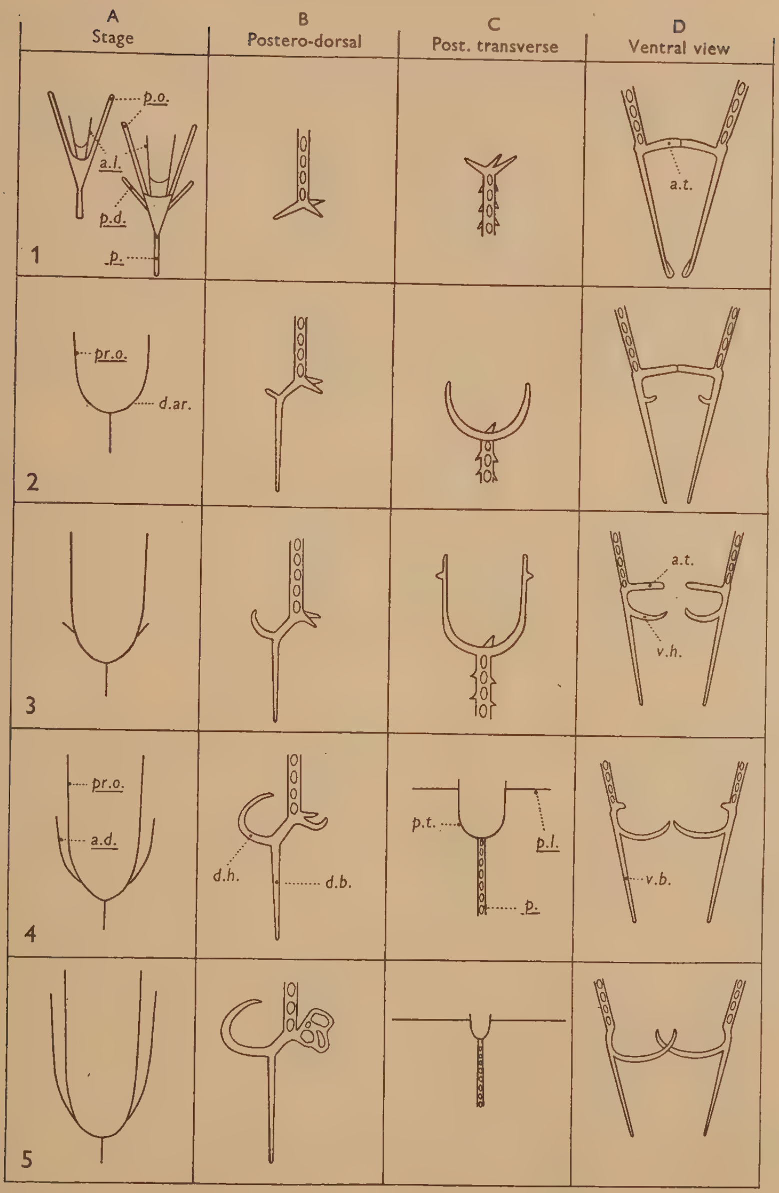
A
Stage
B
Postero-dorsal
C
Post. transverse
D
Ventral view
p.o.
a.l.
p.d.
p.
1
a.t.
pr.o.
d.ar.
2
3
a.t.
v.h.
pr.o.
a.d.
4
d.h.
d.b.
p.t.
p.l.
p.
v.b.
5

Fig. 2. Showing diagrammatically in vertical series the development, through five stages (A), of the postero-dorsal arm rod (B), the posterior transverse rod (C) and the body skeleton as seen in ventral view (D). Abbreviations as in Fig. 1.

The ventral view of the body skeleton is shown in Fig. 2D. In early stage 1 the ventral body rods are usually connected at the posterior end, but usually this soon breaks down. In stage 2 the rudiments of the ventral horizontal rods appear. These greatly increase in size through to stage 5, whilst the anterior transverse rods decrease to become rudiments in stage 4 and indistinguishable in stage 5.

The side views of the body skeleton are shown in Fig. 3. We may take those for *Echinocardium cordatum* to illustrate the sequence. By stage 2 there has been absorption of part of the recurrent rod. The absorption of the recurrent rod and posterior connexion progresses through to stage 4. In stage 3 there is the beginning of the perforated calcareous plate complex which increases through stage 4. By stage 5 there are two separate parts, the postoral arm-body rod system and the antero-lateral arm system. As explained above, the two calcareous plates overlay each other, with that of the postero-dorsal arm in the middle.

It has not been ascertained how these plates develop. It would seem that in stage 4 there is not a single perforated plate as shown in the drawing, but two plates, set at an angle, which arise independently. In *E. flavescens* fingers have been seen to arise from the base of the postoral arm and body rod to form the early stage of the postoral calcareous plate. (This observation is not shown in the drawings.) What is not clear is how much, if any, of the anterior connexion becomes incorporated in the two plates.

THE IDENTIFICATION OF LARVAE

Several distinguishing characters of the various larvae are listed in the descriptions which follow. Experience has shown that it is unwise to depend on any single character for naming a larva. There is a substantial degree of variation in most characters, and identification should usually be based on several indications. The drawings of the side views of body skeletons given in Fig. 3 have been done with the aid of a camera lucida and with the same magnification, but the ventral horizontal rods, which become very long, are not shown in their full length. It may again be said that development is gradual. There is not necessarily any significance in the drawings showing a more advanced degree of development of one species than another at a particular stage. One drawing may be of a larva in an early condition in that stage and the other in a later condition; of the many drawings made of each species, a series has been selected which best shows the sequence of changes.

Fig. 4 has also been prepared with the aid of a camera lucida. The posterior arm is that in stage 3, the complete postero-lateral arm in early stage 4, and the basal part of the same rod in stage 5. The basal parts of the postoral and postero-dorsal arms are in stage 2, but the length of the unfenestrated part, which is the feature illustrated, remains more or less the same throughout the development of a larva and is independent of stage of development.

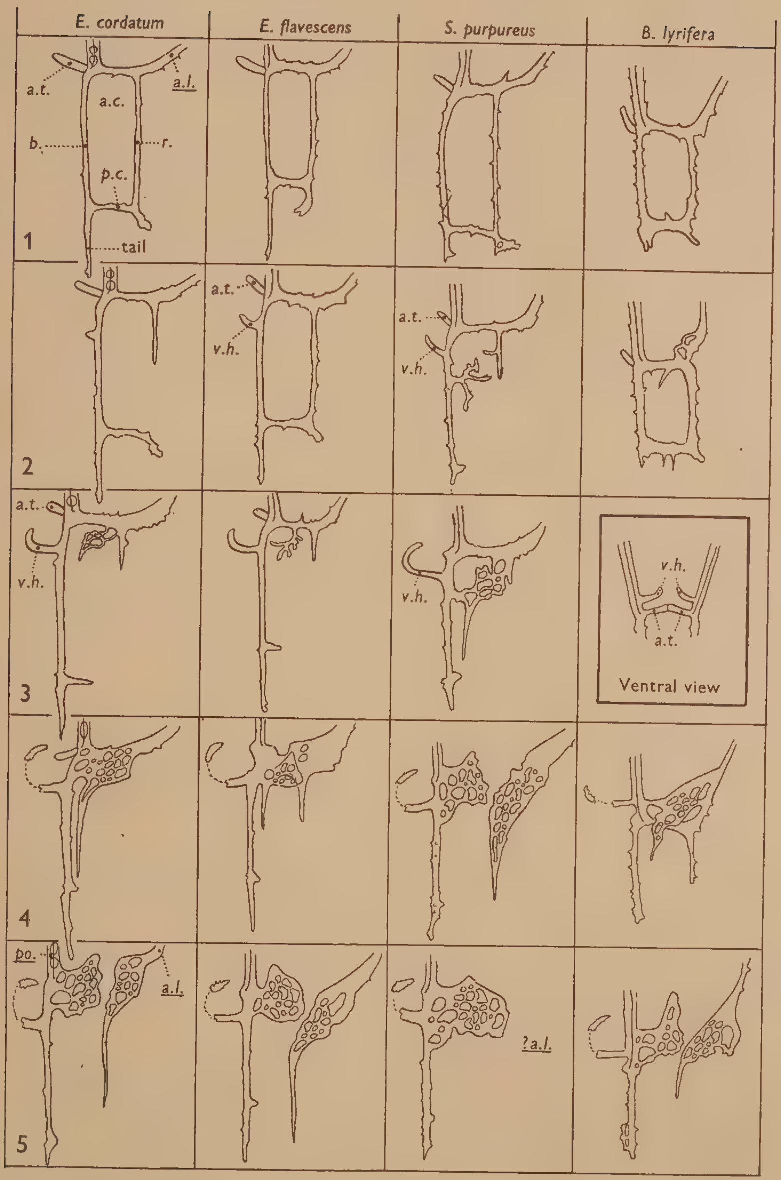
E. cordatum
E. flavescens
S. purpureus
B. lyrifera
a.t.
a.l.
a.c.
b.
r.
p.c.
tail
1
a.t.
v.h.
a.t.
v.h.
2
a.t.
v.h.
v.h.
v.h.
a.t.
Ventral view
3
4
po.
a.l.
?a.l.
5

Fig. 3. The development, through five stages, of the body skeleton of four species, as seen in side view. A diagrammatic ventral view only is given for stage 3 of *B. lyrifera*. Abbreviations as in Fig. 1.

Echinocardium cordatum. The postoral, postero-dorsal, and posterior arms (Fig. 1) are fully fenestrated, unlike those of the other three species. The 'tail' of the body rod is relatively long, a half or more the length of the main body rod. The earliest development of the calcareous plates is off the anterior connexion. The ventral horizontal rod is situated some distance posterior to the base of the postoral arm (and the anterior transverse rod). In the last

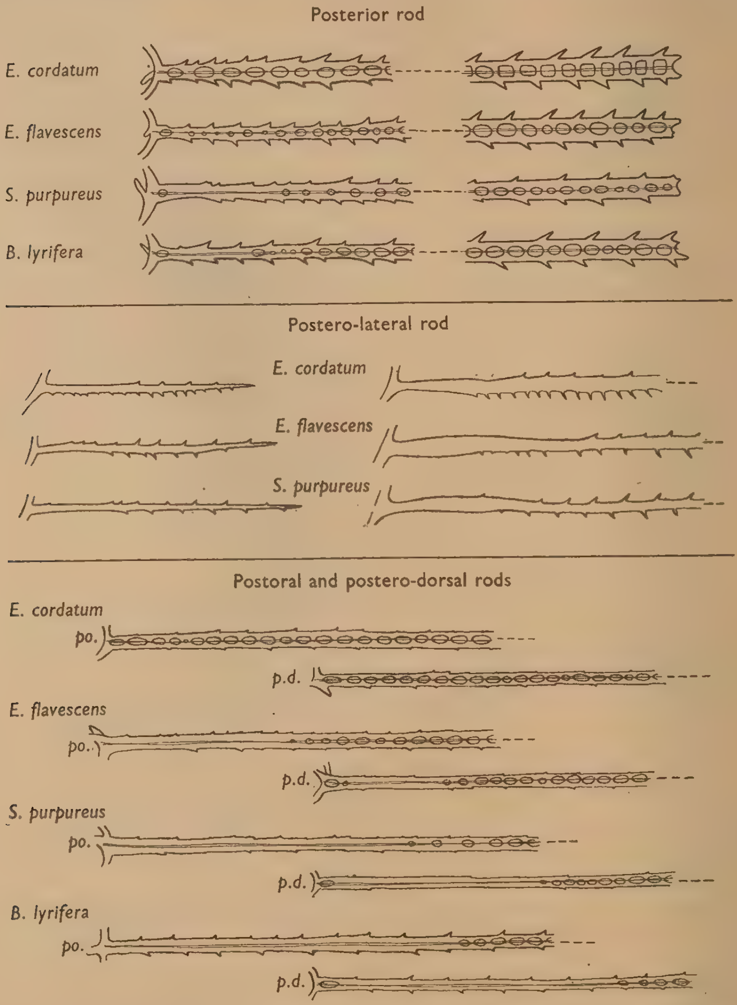
Posterior rod
E. cordatum
E. flavescens
S. purpureus
B. lyrifera
Postero-lateral rod
E. cordatum
E. flavescens
S. purpureus
Postoral and postero-dorsal rods
E. cordatum
po.
p.d.
E. flavescens
po.
p.d.
S. purpureus
po.
p.d.
B. lyrifera
po.
p.d.

Fig. 4. Typical examples of the posterior, postero-lateral, postoral and postero-dorsal arm rods of four species. Abbreviations: *po.*, postoral; *p.d.*, postero-dorsal.

stage, the support for the posterior edge of the calcareous plate of the postoral arm is markedly anterior to the place of origin of the ventral horizontal rod. The postero-lateral arm is very spiny; there are approximately twice as many spines on the posterior edge as on the anterior.

Echinocardium flavescens. The posterior arm is, with rare exceptions, fully fenestrated. The fenestrations in the proximal part may be fairly large or very small; rarely a section may appear to be unfenestrated. The proximal part of the postoral arm is unfenestrated to a variable degree (Fig. 5), the great majority (forty-seven out of fifty measurements) to a length of 0·10–0·17 mm. inclusive. The proximal part of the postero-dorsal is unfenestrated, apart from a single basal fenestration. Along the unfenestrated part there may occasionally be an isolated fenestration. The length of the unfenestrated part, from the base of the arm to the beginning of the regular series of fenestrations, is variable (Fig. 5), twenty measurements falling into the range 0–0·13 mm.

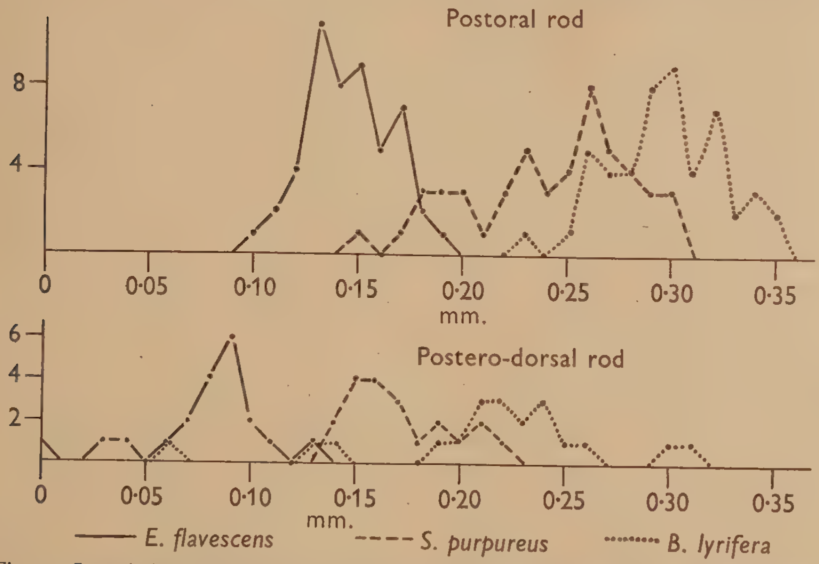

Fig. 5. Length-frequency distribution of the unfenestrated portion of the postoral arm (fifty measurements in each species) and the postero-dorsal arm (twenty measurements in each species) of *Echinocardium flavescens*, *Spatangus purpureus* and *Brissopsis lyrifera*.

The 'tail' of the body rod, relative to the full length of the body rod, is as long as that of *E. cordatum*. The earliest development of the calcareous plates is off the anterior connexion. The ventral horizontal rod is situated nearer to the base of the postoral arm than in *E. cordatum*, and the support for the posterior edge of the postoral calcareous plate is more or less at the same level as the ventral horizontal rod, or very slightly anterior. The postero-lateral arm is not so spiny as in *E. cordatum*; over the greater part the spines on the posterior and anterior edges regularly alternate.

Spatangus purpureus. The proximal part of the posterior arm is unfenestrated to a variable length, but commonly about 0·1 mm., apart from a single basal fenestration. In occasional larvae the variation in degree of fenestration may overlap that of *Echinocardium flavescens*. The postoral arm is unfenestrated to a length substantially greater than in *E. flavescens*, forty-eight out of fifty measurements falling into the range 0·18–0·3 mm. inclusive (Fig. 5). The proximal part of the postero-dorsal arm is unfenestrated except for a single basal fenestration and, occasionally, isolated fenestrations. Twenty measurements of the length of the unfenestrated portion, to the beginning of the series of fenestrations, fall into the range 0·14–0·22 mm., being greater than corresponding measurements for *E. flavescens* (Fig. 5).

The 'tail' of the body rod is relatively shorter than in the two *Echinocardium* species. The body rods are distinctly more spiny than in *Echinocardium*. The first development of the calcareous plate takes place from the body rod. The horizontal rod arises close to the base of the postoral arm. The support for the posterior edge of the postoral calcareous plate is distinctly posterior to the place of origin of the horizontal rod. All the calcareous plates develop at an earlier stage than in *Echinocardium*, and are larger. The postero-lateral arm is less spiny than in *E. flavescens*. Over the greater part, the posterior and anterior spines regularly alternate.

Brissopsis lyrifera. The proximal portions of the posterior, postoral and postero-dorsal arms are unfenestrated, normally to a greater length than in *Spatangus*, but with much variability (Fig. 5). The postoral and postero-dorsal arms are distinctly more spiny than in the other three species.

The body rods are shorter, thicker and much more spiny than in the other three species. There is no distinctive 'tail'. In the earliest stages there is an extension beyond the posterior connexion, but this is merely the basal part of the connexion which meets its partner in the median line.

The earliest development of the calcareous plates takes place off the basal part of the antero-lateral arms. The ventral horizontal rod emerges off the postoral arm proper, and *anterior* to the base of the arm (and anterior transverse rod). There is no postero-lateral arm even in the last stage.

DISCUSSION

To my knowledge, the most satisfactory account of a spatangoid skeleton is that by Ohshima (1921) of the larva of *Spatangus purpureus*. Some developmental stages were, however, not available to him, so his account is incomplete and significantly in error. He was unable to recognize that the anterior transverse rod is replaced during development by the ventral horizontal rod. Krohn (1853) gave a ventral view of a larva, corresponding to stage 3 in

Fig. 2, in which both transverse and horizontal rods are present, but did not comment on this. Ohshima (1921) judged that Krohn's drawing was either a misrepresentation or of an abnormal larva. It is, on the contrary, a normal stage of development.

I have not followed the development of the only clypeastroid known in British waters, *Echinocyamus pusillus* (O. Fr. Müller), in any detail, since there is no difficulty in identifying the larva. It is certain, however, that the same replacement of the transverse rod by the horizontal rod occurs, though this was not appreciated by Théel (1892), who described the larva in great detail. Onoda (1938) described the replacement as occurring in the larvae of *Echinarachnius brevis* Ikeda and *Astriclypeus manni* Verrill. Mortensen (1921, p. 231), on the other hand, refers only to the reduplication of the ventral transverse rod in clypeastroids. Later, he described a replacement as occurring in *Fibularia craniolis* (Leske) but called it 'a curious feature' (Mortensen, 1937). Despite this, it is probable that the replacement is a normal feature in clypeastroid larvae, as well as in spatangoid larvae.

Mortensen (1921, p. 202) pointed out that spatangoid larvae may be divided into two groups, one group containing species with postero-lateral arms and the other group those species without. This grouping has the disadvantage that young larvae (and of several spatangoid species only the young larvae have been described) cannot be assigned to a group, for the postero-lateral arm, if present in a species, is rather a late development. A review of the literature on spatangoid larvae suggests that the same grouping will result if the presence or absence of a 'tail' to the body rod is taken as the dividing character; this character would allow the division of very young larvae.

The larva of *Brissopsis lyrifera* is a representative of the group without postero-lateral arms and without a 'tail' to the body rod. A particularly striking characteristic of the larva is the development of the ventral horizontal rod anterior to the transverse rod. In the larvae of *Echinocardium* and *Spatangus*, which are representative of the other group, the ventral horizontal rod develops posterior to the transverse rod.

The larvae of *Echinocardium* and *Spatangus* are very similar; the only difference observed which is likely to be of generic value is the mode of formation of the calcareous plate complex. More information about this process is required than is now available. That there is a basic difference is indicated by the position where the early development occurs, in the region of the anterior connexion in *Echinocardium*, off the body rods in *Spatangus*. It is noteworthy that *Brissopsis* differs from both *Echinocardium* and *Spatangus* in this respect also.

It is unfortunate that the larva of *Echinocardium pennatifidum* remains unknown, for it may prohibit certain identification of *E. cordatum* or *E. flavescens* in the plankton from some places. Arguing that the characters which are common to the larvae of the two latter species are generic characters, it is likely that the larva of *E. pennatifidum* has postero-lateral arms and a 'tail' to the body rod, the ventral horizontal rod is posterior to the anterior transverse rod, and that the earliest development of the plate complex is in the region of the anterior connexion.

It is not unlikely that the larva of *E. pennatifidum* was present in my material. One of two types of larvae observed may, in fact, be of this species. A single larva observed had all features indicating *E. flavescens* except that the arms were fully fenestrated. This larva was probably outside the range of variation of either *E. cordatum* or *E. flavescens*, but it may have been a hybrid form rather than the unknown larva of *E. pennatifidum*. Several larvae suspected of being *E. cordatum* had postero-lateral arms which were more spiny than usual in undoubted *E. cordatum* larvae. In the main part of the arm in *E. cordatum*, the ratio of posterior spines to anterior spines is approximately 2:1; but in these particular larvae the ratio was greater than 3:1, due to more crowded spines in the posterior edge. These larvae may have been within the range of variation of *E. cordatum* larvae.

It has been usual to indicate the length of the arms, particularly of the posterior process, in describing echinoplutei. Little attention has been given to this point in the present investigation, partly because the available material has not been very suitable, and partly because it has appeared that too much attention to this may be misleading. To be really useful it is required to know the length at each stage and the variation in length. There is good reason for believing the variation in length is considerable. Mortensen (1927) says of *E. cordatum* that the posterior process is 'exceedingly variable in length, sometimes a mere short stump, sometimes as long as the postoral arms'. The larva of *Brissopsis* is described as having a short posterior process (Mortensen, 1920). This is true for larvae which I have seen which had been taken from the Clyde, but some undoubted *Brissopsis* larvae from south-west of Ireland had processes 2 mm. long, that is, longer than that given in Mortensen's (1913) drawing of the larva of *Spatangus purpureus*, whose process is said to be 'exceedingly long'. It would seem at least possible that the variation in the *Spatangus* process is also considerable. On the whole, the posterior process of *E. flavescens* appears to be longer than that of *E. cordatum*.

There is a difficulty in describing echinoplutei, in that the variations are on the whole considerable. The length of the processes and the extent of development of calcareous plates would seem in part determined by the environment. Hörstadius (1940) concluded, following an investigation of echinoid hybrids, that the amount of skeleton produced is increased by higher temperatures. Tennent (1929) pointed out that slight changes in the environmental medium may induce considerable changes in the character of the echinoderm larval skeleton. The length of the unfenestrated portion of the fenestrated arms is also very variable (Fig. 5). The position of the postoral calcareous plate in relation to the ventral horizontal rod, and the region of early development of the plate complex should, however, prove to be good specific and generic characters.

SUMMARY

An account is given of the general structure of the body skeleton of the spatangid larva. A considerable change occurs in the skeleton as the larva increases in size, some parts being absorbed and new parts appearing. Of particular interest is the replacement of the anterior transverse rod by the ventral horizontal rod.

The skeletons of larvae, young stages to late stages, of *Echinocardium cordatum*, *E. flavescens*, *Spatangus purpureus* and *Brissopsis lyrifera* are described. The larvae of *Echinocardium* and *Spatangus* are, in essentials, very similar; that of *Brissopsis* differs greatly from them. A striking difference is that in *Brissopsis* the ventral horizontal rod arises from the postoral arm and anterior to the anterior transverse rod, in the other two genera it arises from the body rod posterior to the anterior transverse rod.

The replacement of the anterior transverse rod by a ventral horizontal rod is probably normal in clypeastroid larvae also.

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J. Mar. biol. Ass. U.K. (1953) 32, 491–495
Printed in Great Britain

CELLULOLYTIC ACTIVITY IN THE LAMELLIBRANCH CRYSTALLINE STYLE

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Lavine (1946) gives the first report of a lamellibranch style cellulase. Whilst investigating the style amylases of *Mactra* and *Mya*, he dialysed aqueous solutions of crystalline style material through Visking casing (regenerated cellulose) and observed that the casing eroded away. Strips of Visking casing immersed in style solution were also dissolved, but filter paper was little affected. Up till then the existence of cellulolytic activity in the lamellibranch style had been denied by Yonge (1926) and Fox *et al.* (1936), for though Fox did obtain some very slight evidence of cellulose utilization, he dismissed it as negligible.

However, the lamellibranch diet seems to consist mainly of dinoflagellates (in fact the sea mussel *Mytilus californianus* is a selective feeder, rejecting diatoms and ingesting dinoflagellates, see Fox *et al.*, 1936), and since many of these latter organisms are reputed to possess a cellulose cell wall or cuticle, their assimilation would seem to require a cellulase. Cellulases have, of course, been reported in many other Mollusca, e.g. *Pterocera* (Yonge, 1932), *Xylophaga* (Purchon, 1941), *Teredo* (Potts, 1923), and the gastric cellulase of *Helix* has been known for many years.

It seemed, in view of Lavine's findings, that the absence of cellulolytic activity previously reported in the lamellibranchs might have been due to the use of an unsuitable substrate. Boswell (1941) quotes evidence that regenerated cellulose is much more easily utilized in the cellulolytic processes of microorganisms. In view of the report by Yonge (1926) of the presence of active spirochaetes in the crystalline style of the oyster, *Ostrea edulis*, it seemed worth while to make use of this lamellibranch, and also of the mussel, *Mytilus edulis*, in an investigation of the possible presence of a style cellulase with an action upon regenerated cellulose.

Acknowledgements are due to Dr C. P. Spencer for much advice and encouragement, and to Dr J. E. Morton for suggesting the project in the first instance. This work was done at the Plymouth Laboratory while I held a Colonial Office Studentship.

METHODS

The cellulose substrate used was in the form of a finely divided suspension of filter-paper, prepared by the method of Scales as given by Stephenson (1939), The stability of the suspension was found to be improved by passage through an 'Ormerod' emulsifier. In view of the method used (precipitation from 62·5 %, v/v, sulphuric acid) the following tests for possible breakdown products of cellulose were carried out: (*a*) Reducing sugars. Qualitatively by Benedict's reagent and the picramate test. Quantitatively by Somogyi's method (1930). (*b*) Amyloids. Iodine coloration test.

No reducing activity was obtained either in the suspension itself or in filtrates from it, subjected to acid hydrolysis. There were no amyloids present, and no cellulose could be detected in filtrates of the suspension (chlor-zinc-iodine test). The suspension gave a green ring in the Molisch test, changing to the more usual violet coloration after about 15 min. Filtrates gave no Molisch reaction at all.

Utilization of cellulose was followed turbidimetrically in a Harvey absorptiometer (Harvey, 1948), and the units of 'turbidity' given in Tables I and II are those of the absorptiometer scale—which are logarithmic, being based on Beer's Law. The data show that the turbidity of the cellulose suspension alone remains constant over the periods of time used in the experiments, and the turbidity of the suspension was found to be proportional to the amount of cellulose present (cf. Stadi & Riggs, 1943).

The style solutions were made by dissolving crystalline style material from *Mytilus* and *Ostrea* in citrate-phosphate buffer at various pH values in the region of 5·5. Preliminary experiments showed that the optimum pH of the cellulolytic activity lay in this region, which approximates to the pH of the stomach fluid in *Ostrea* (Yonge, 1926). The cellulose in later experiments was also suspended in citrate-phosphate buffer. All experiments were conducted at laboratory temperature, which varied between 15 and 19° C.

RESULTS

Boiling tubes were set up in series, each containing an equal volume of reaction mixture. A typical experiment was as follows, using style material from *O. edulis*.

Tubes A and B, 5 ml. buffer + 3 ml. cellulose suspension.
Tubes C, E and F, 5 ml. style solution + 3 ml. cellulose suspension.

Tubes B and C were boiled for 3 min. to act as controls. The turbidities of the five tubes were measured at intervals and are given in Table I as absorptiometer scale readings.

TABLE I

	Hours								
Tube	0	1	2	3	4	6	7	8	20
A	275	270	269	270	262	270	270	265	268
B	275	270	268	270	262	268	272	269	270
C	408	406	410	406	404	400	402	408	409
E	235	225	207	200	187	176	177	171	146
F	238	223	213	194	184	173	168	164	142

After this experiment, 5 ml. samples from tubes A, B, E and F were taken (shaking well to disperse the suspension as uniformly as possible). Each sample was centrifuged, decanted, and washed, three times, and the residual cellulose then dried to constant weight. The weights are shown below:

Tube	Residual cellulose (mg.)	Tube	Residual cellulose (mg.)
A (control)	14·9	E (active)	10·2
B (control)	15·0	F (active)	8·6

The activity is clearly thermo-labile.

In the case of experiments using material from *Mytilus edulis*, a similar decline in turbidity in tubes containing unboiled style solution and cellulose occurred, but activity was less than with style material from *Ostrea*. The styles from the two animals differ in appearance, and Berkeley (1935) has shown that qualitative differences in the glyco-proteins, at least, of various lamellibranch styles do occur. Since it might have been that the cellulose utilization was merely due to some of the numerous bacteria inhabiting the style, various preservative agents were used in later experiments. 'Merthiolate' (1 in 200,000) and chloroform (0·5–8 ml. of active preparation) did not appear to inhibit cellulose utilization. Xylene and toluene (also 0·5–8 ml. of active preparation) made turbidity measurements impracticable, but after one experiment, using *Mytilus* material, and toluene, the residual cellulose in each of three tubes after 20 hr. activity was collected, washed, dried and weighed. The weights are given below:

Tube	Residual cellulose (mg.)
Control	14·3
Preparation with toluene	8·9
Preparation without toluene	8·6

Thus toluene, at least, does not abolish cellulose utilization.

It was found that reducing substances appeared in active preparations as cellulose disappeared, and in some experiments the increase in such reducing activity, with time, was followed.

In one such experiment, five tubes were set up containing cellulose suspension in buffer and *Mytilus* style material, also in buffer. Relevant controls were set up as before, but this time included a tube with style solution only, to compensate for reducing activity in the style itself. This proved to be a necessary precaution.

The turbidities of tubes containing the whole system showed a regular decline, whilst those of the controls remained constant. After various periods of time, single tubes were withdrawn and activity stopped by the addition of sodium hydroxide solution to a pH above 8·5 (phenol red). The reaction mixtures were filtered and the filtrates retained. Estimation of reducing activity was effected by the Somogyi method (Somogyi, 1930), on 5 ml.

samples from two tubes before, and all five tubes after, acid hydrolysis. This hydrolysis was carried out by heating the samples for 10 hr. at 90° C., after adding five drops of concentrated sulphuric acid. The reducing activity is given below, corrected for reagent and style solution reducing substances, and expressed as milligrams of glucose per millilitre of reaction mixture.

Incubation time (hr.)	Reducing substances	
	Before acid hydrolysis	After acid hydrolysis
0	Nil	Nil
6½	0·051	0·066
19	—	0·116
25	—	0·144
43	0·072	0·166

It will be seen that substances able to reduce the Somogyi reagent are found in the *Mytilus* style preparation, but that the large increase in the reducing power of filtrates after acid hydrolysis suggests that the principal immediate products of cellulose breakdown are soluble compounds with little reducing power.

When active tubes were evacuated or tightly stoppered, the activity was slowed down or stopped. Using style material from *Mytilus*, five experimental tubes were set up (Table II). Control tube B showed no change with time. Boiling the control tube 1 increased the turbidity which then showed no further change with time. After 5 hr. the active tube 3 was tightly stoppered which prevented further reduction of turbidity. In the other two active tubes reduction continued until the end of the experiment.

TABLE II. TURBIDITY, AS LOGARITHMIC SCALE READINGS OF THE ABSORPTIOMETER

Tube	Hours								
	0	1	2	3	5	7	19	22	25
B (control)	363	364	366	365	370	367	367	370	370
1 (control)	662	662	662	663	662	661	662	662	662
3 (active)	432	402	390	384	375	377	370	376	374
4 (active)	417	392	374	371	358	352	327	322	312
5 (active)	412	382	367	362	352	350	314	314	314

From this investigation and from Berkeley's (1933 *a*, *b*) work on style oxidase, presence of glucosone (2-keto-glucose) was expected but neither glucosone nor glucose could be detected, no keto groups could be demonstrated by the Rothera or nitro-prusside tests.

On warming protein-free filtrates of reaction mixtures with phenyl hydrazine hydrochloride, in 2N-hydrochloric acid, a phenyl hydrazone of colourless pinnate crystals could be obtained. After acid hydrolysis, filtrates gave an ozazone resembling glucosazone, and having a decomposition point at 205° C. Further identification of the products of this cellulose utilizing system was not possible in the limited time available.

This investigation extends Lavine's findings of cellulase in *Mactra* and *Mya* to *Ostrea* and *Mytilus*. It has not established an actual relation between the cellulolytic action of the style and the presence of spirochaetes, and in *Mytilus* the bacterial population of the style is not yet known. In view, however, of the known ability of some groups of spirochaetes (see Walker & Warren, 1938) to hydrolyse cellulose to simpler carbohydrates, a connexion between style spirochaetes and cellulolytic action remains an interesting possibility. Morton (1952) discusses this point more fully.

SUMMARY

A method of demonstrating cellulose utilization in biological systems by measurement of the turbidity of a cellulose suspension in a photo-electric absorptiometer is described. The crystalline styles of *Ostrea edulis* and *Mytilus edulis* appear to contain a cellulolytic factor as yet uncharacterized.

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J. Mar. biol. Ass. U.K. (1953) 32, 497–520
Printed in Great Britain

A PRELIMINARY CHECK-LIST OF BRITISH MARINE ALGAE

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This preliminary list of some of the classes of the algae has been prepared, mainly for the use of British phycologists, to form a basis on which to work for the eventual compilation of a complete check and locality list of all species of British marine algae. The compiler would be grateful for all additions and corrections to this list, and for records of marine species from the algal classes not included in the present list.

The classes of algae treated in this list are the Chlorophyceae (53 gen., 156 spp.), Xanthophyceae (7 gen., 15 spp.), Chrysophyceae (15 gen., 22 spp.), Phaeophyceae (96 gen., 199 spp.), Rhodophyceae (126 gen., 324 spp.), and Cyanophyceae (39 gen., 125 spp.). The genera are listed alphabetically under the family and the species alphabetically under the genus. If a name has been changed from that given in Newton (1931), the name in Newton is added, in brackets, below. Names preceded by a question mark appear to need revision for nomenclatural or taxonomic reasons. Most of the so-called varieties listed are very probably merely habitat forms of the species, but further investigation is needed before new combinations are made for them. Notes are given at the end of each class, the number in brackets following a name in the list refers to these notes.

The compiler is grateful to Mr R. Ross and Miss Linda Newton, of the British Museum, and to Miss C. I. Dickinson, of the Herbarium, Kew, for their help over a number of problems. She is also grateful to Miss D. Ballantine for her assistance in checking through some of the literature for marine algal records. Sincere thanks are due to Dr P. Kornmann and to Dr C. Bliding for information. The compiler is also most grateful to Mr F. S. Russell, F.R.S., and to Mr G. M. Spooner for their encouragement and for suggestions as to the form the list should take.

CHLOROPHYCEAE

VOLVOCALES

Chlamydomonadaceae
Brachiomonas Bohlin, 1897.
simplex Hazen
submarina Bohlin
? *westiana* Pascher
Carteria Diesing, 1866.
excavata Mass. ex Carter
Chlamydomonas Ehrenberg, 1833.
brachyura G. S. West
microplankton Rke.
quadrilobata Carter
Platymonas G. S. West, 1916.
apiculata Butcher
contracta Carter
tetrathele G. S. West
Polyblepharidaceae
Bipedinomonas Carter, 1937.
pyriformis Carter
rotunda Carter
Dunaliella Teodoresco, 1905.
salina (Dunal) Teodor.
Heteromastix Korshikov, 1923.
minuta Carter
Pyramimonas Schmarda, 1850. (3)
angulata Carter
grossii Parke
obovata Carter
octociliata Carter
olivacea Carter

Chlorodendraceae
Prasinocladus Kuckuck, 1894.
lubricus Kuck.

CHLOROCOCCALES

Chlorococcaceae
Characium A. Braun, 1849.
? *marinum* Kjellm. (1)
Chlorochytrium Cohn, 1872.
cohnii Wright
dermatocolax Rke.
facciolae (Borzi) Bristol
immersum Masse
inclusum Kjellm.
moorei Gardner
willei Printz
Codiolum A. Braun, 1855. (2)
petrocelidis Kuck.
? *pusillum* (Lyngb.) Kjellm.
Sykidion Wright, 1881.
dyeri Wright

Chlorellaceae
Chlorella Beijerinck, 1890.
marina Butcher
ovalis Butcher
salina Butcher
stigmatophora Butcher
Nannochloris Naumann, 1919. (3)
atomus Butcher
maculatus Butcher
Trebouxia de Puymaly, 1924.
humicola (Treboux) Puymaly

ULOTRICHALES

Ulotrichaceae
Stichococcus Nägeli, 1849.
cylindricus Butcher
Ulothrix Kützing, 1833. (4)
consociata Wille
flacca (Dillw.) Thur.
pseudoflacca Wille
subflaccida Wille
(? *implexa* Kütz.)

Monostromaceae
Monostroma Thuret, 1854. (5)
crepidinum Farl.
fuscum (Post. et Rupr.) Wittr.
(*blyttii* (Aresch.) Wittr.)
grevillei (Thur.) Wittr.
oxyspermum (Kütz.) Doty
(*laceratum* Thur.)
(*latissimum* (Kütz.) Wittr.)
(*orbiculatum* Thur.)
(*quaternarium* (Kütz.) Desm.)
(*wittrockii* Born.)
undulatum Wittr.

Ulvaceae
Blidingia Kylin, 1947.
minima (Näg.) Kylin
(*Enteromorpha minima* Näg.)
(*Enteromorpha micrococca* Kütz.)
Capsosiphon Gobi, 1879. (6)
fulvescens (Ag.) Setch. et Gardn.
(*C. aureolus* (Ag.) Gobi)
Enteromorpha Link, 1820. (7)
ahlneriana Bliding
clathrata (Roth) Grev.
compressa (L.) Grev.
intestinalis (L.) Link
? *lingulata* J. Ag.
linza (L.) J. Ag.
? *marginata* J. Ag.
prolifera (Müll.) J. Ag.
ralfsii Harv.
ramulosa (Sm.) Hook.
torta (Mert.) Reinb.
? *usneoides* (Bonnem.) J. Ag.
Percursaria Bory, 1823.
percursa (Ag.) Bory
Ulva Linnaeus, 1753.
? *lactuca* L.
var. ? *lactuca*
var. ? *latissima* (L.) D.C.
var. ? *rigida* (Ag.) Le Jol.

PRASIOLALES

Prasiolaceae
GAYELLA Kolderup Rosenvinge, 1893.
polyrhiza K. Rosenv.
PRASIOLA Agardh, 1822.
crispa (Lightf.) Menegh.
stipitata Suhr

CHAETOPHORALES

Chaetophoraceae
ACROCHAETE Pringsheim, 1862.
parasitica Oltm.
repens Pringsh.
BOLBOCOLEON Pringsheim, 1862.
piliferum Pringsh.
CHAETOBOLUS Kolderup Rosenvinge, 1893.
gibbus K. Rosenv. (8)
ECTOCHAETE (Huber) Wille, 1909. (9)
leptochaete (Huber) Wille
(*Endoderma leptochaete* Huber)
wittrockii (Wille) Kylin
(*Endoderma wittrockii* Wille)
ENTOCLADIA Reinke, 1879.
perforans (Huber) Levring
viridis Rke.
(*Endoderma viride* Lagerh.)
EPICLADIA Reinke, 1889.
flustrae Rke.
(*Endoderma flustrae* Batt.)
var. *flustrae*
var. ? *phillipsii* Batt.
OCHLOCHAETE Thwaites ex Harvey, 1849. (8)
ferox Huber
hystrix Thwaites
PHAEOPHILA Hauck, 1876.
dendroides (Crn.) Batt.
engleri Rke.
PILINIA Kützing, 1843.
maritima (Kjellm.) K. Rosenv.
rimosa Kütz.
PRINGSHEIMIELLA v. Hoehnel, 1920.
scutata (Rke.) Marchew.
(*Pringsheimia scutata* Rke.)
PROTODERMA Kützing emend. Borzi, 1895
marinum Rke.
PSEUDOPRINGSHEIMIA Wille, 1909.
confluens (K. Rosenv.) Wille
(*Ulvella confluens* K. Rosenv.)
fucicola (K. Rosenv.) Wille (10)
(*Ulvella fucicola* K. Rosenv.)
PSEUDULVELLA Wille, 1909.
applanata Setch. et Gardn.
ULVELLA Crouan, 1859.
lens Crn.

Trentepohliaceae
? GOMONTIA Bornet et Flahault, 1888. (11)
? *manxiana* Chodat
? *polyrhiza* (Lagerh.) Born. et Flah.

TELLAMIA Batters, 1895.
contorta Batt.
? *intricata* Batt.

CLADOPHORALES

Cladophoraceae (12)
ACROSIPHONIA J. Agardh; sensu Wille, 1909.
centralis (Lyngb.) Kjellm. (13)
(*Cladophora* (*Spongomorpha*) *arcta* (Dillw.) Kütz.)
CHAETOMORPHA Kützing, 1845.
? *aerea* (Dillw.) Kütz.
capillaris (Kütz.) Børg. (14)
crassa (Ag.) Kütz.
linum (Müll.) Kütz.
? *litorea* Cooke
melagonium (Web. et Mohr) Kütz.
CLADOPHORA Kützing, 1843.
albida (Huds.) Kütz.
var. *albida*
var. *refracta* (Harv.) Thur.
? *arctiuscula* Kütz.
? *balliana* Harv.
brownii (Dillw.) Harv.
cornea Kütz.
var. *verticillata* Kütz.
? *corymbifera* Kütz. (17)
corynarthra Kütz.
var. *spinescens* Batt.
expansa (Mert.) Kütz.
? *falcata* Harv.
? *flexuosa* (Griff.) Harv.
fracta (Müll.) Kütz. (15)
glaucescens (Griff.) Harv.
gracilis (Griff.) Kütz.
hirta Kütz.
hutchinsiae (Dillw.) Kütz.
var. *hutchinsiae*
var. *distans* (Ag.) Kütz. (16)
var. ? *divaricata* Harv.
macallana Harv.
magdalenae Harv.
pachyderma (Kjellm.) Brand
var. *tenuior* (Børg.) Brand
pellucida (Huds.) Kütz.
var. *pellucida*
var. ? *comosa* Kütz.
var. ? *cristata* Kütz.
var. ? *curvata* Kütz.
prolifera (Roth) Kütz.
ramosissima (Drap.) Kütz.
f. *humilis* (Kütz.) Hamel
(*C. neesiorum* Kütz. var. *humili* Batt.)
rectangularis (Griff.) Harv.
f. *rectangularis*
f. *hispida* Kütz.
f. *horrida* Kütz.
f. *subnuda* Kütz.
? *refracta* (Roth) Kütz. (17)
repens (J. Ag.) Harv.
rudolphiana (Ag.) Harv.

- CLADOPHORA (*cont.*)
  - *rupestris* (L.) Kütz.
    - f. *rupestris*
    - f. ? *distorta* (Harv.)
    - f. *nuda* (Harv.) Hamel
  - ? *sericea* (Huds.) Kütz. (18)
  - *sonderi* Kütz.
  - *stolonifera* (Kjellm.) Batt.
  - ? *traillii* (Batt.) Batt.
  - ? *trichocoma* Kütz.
  - *utriculosa* Kütz.
    - var. *utriculosa*
    - var. ? *diffusa* Hauck
- RHIZOCLONIUM Kützing, 1843.
  - *arenosum* (Carm.) Kütz.
  - ? *hieroglyphicum* (Ag.) Kütz.
  - *implexum* (Dillw.) Kütz.
  - ? *kerneri* Stockm.
  - *kochianum* Kütz.
  - *riparium* (Roth) Harv.
  - *tortuosum* (Dillw.) Kütz. (19)
- SPONGOMORPHA Kützing, 1843.
  - *bombycina* (Kjellm.) Wille
  - *lanosa* (Roth) Kütz.
    - var. *lanosa*
    - var. ? *uncialis* (Müll.)
  - *pallida* (Kjellm.) Kylin
- UROSPORA Areschoug, 1866.
  - *bangioides* (Harv.) Holm. et Batt.
  - *penicilliformis* (Roth) Aresch. (2) (*isogona* (Sm.) Batt.) sporophyte *Codiolum gregarium* A.Br.
  - *speciosa* (Carm.) Leblond ex Hamel (*Ulothrix speciosa* (Carm.) Kütz.)
  - *wormskioldii* (Mert.) K. Rosenv. (*collabens* (Ag.) Holm. et Batt.)

? SIPHONALES

Caulerpaceae

- BRYOPSIS Lamouroux, 1809.
  - *hypnoides* Lamour.
  - *plumosa* (Huds.) Ag.
    - var. *plumosa*
    - var. ? *nuda* Holm.
    - var. ? *subsimplex* Holm. et Batt.

Derbesiaceae

- DERBESIA Solier, 1847. (20)
  - *marina* (Lyngb.) Kjellm. gametophyte = *Halicystis ovalis* (Lyngb.) Aresch.
  - *tenuissima* (De Not.) Crn.

Codiaceae

- CODIUM Stackhouse, 1797.
  - *adhaerens* (Cabr.) Ag.
  - ? *amphibium* Moore
  - *bursa* (L.) Ag.
  - ? *fragile* (Sur.) Hariot
    - f. *atlanticum* (Cotton) Levring (*tomentosum* var. *atlanticum* Cotton)
  - ? *tomentosum* Stackh.

Chaetosiphonaceae

- BLASTOPHYSA Reinke, 1888.
  - *rhizopus* Rke.

Phyllosiphonaceae

- OSTREOBIUM Bornet et Flahault, 1889.
  - *quekettii* Born. et Flah.

Notes on Chlorophyceae

(1) Not an independent species according to Levring (1937)—a *Cladophora* sporeling.

(2) *C. gregarium* A.Br., diploid generation of *Urospora penicilliformis*; *C. petrocelidis*, probably an independent form.

(3) Systematic position uncertain; see Chadefaud (1941) on *Pyramimonas* Schmarda.

(4) British species of *Ulothrix* require revision.

(5) British species in urgent need of revision: see Hamel (1931) for species included under *M. oxycoccum* (Kütz.) Thur. = *M. oxyspermum* (Kütz.) Doty.

(6) Chapman (1952) advocates the removal of the genus *Capsosiphon* from the Ulvaceae to separate family, Capsosiphonaceae.

(7) Until British material belonging to this genus has been fully investigated on the lines of the work of Bliding, the limits of the species occurring in Britain cannot be assessed.

Dr C. Bliding has checked the list of species personally, and has very kindly allowed me to publish his records of *E. ahlneriana* for the British coast. He reports:

> 'During an excursion to Anglesey, North Wales, in July 1953, I found *Enteromorpha Ahlneriana* Bliding in the intertidal zone at two different localities, Rhosneigr and Newborough.
>
> 'At Rhosneigr the main axes of the thalli were often very broad with few branches, as in Typus III (Bliding, 1944, p. 343, fig. 16). The material from Newborough, however, was more branched and the axes of the plants, as well as the branches, were more slender.
>
> '*E. Ahlneriana* has so far been recorded only from the west coast of Sweden and from the Baltic Sea. In the Baltic this species is very abundant.' (Carl Bliding, Plymouth, 17 July 1953.)

(8) *Chaetobolus gibbus*, *Ochlochaete ferox* and *O. hystrix*—possibly forms of one species, see Waern (1952).

(9) *Endoderma-Ectochaete-Entocladia-Epicladia* complex—British material needs revision.

(10) *Ulvella fucicola* var. *globosa* Batt.; before making new combination under *Pseudopringsheimia* further investigation seems desirable.

(11) If Kylin (1935) is right *Gomontia* is a name of a taxon derived from two entirely discordant elements and should perhaps be rejected (Art. 76, Lanjouw, 1952).

(12) The British species of all genera in this family are in urgent need of revision.

(13) Part at least, if not all, material recorded for Britain under *Cl.* (*Spongomorpha*) *arcta* should be referred to *Acrosiphonia centralis* (Lyngb.) Kjellm.

(14) = '*Conferva tortuosa* J. Ag.', Harv., *Phyc. Brit.* 54A, *Chaetomorpha tortuosa* Kütz., in Newton, 1931, p. 91 (Chapman, 1939).

(15) British forms referred to this species are in need of revision.

(16) It is generally considered that the alga described and figured by Harvey, *Phyc. Brit.*, 130, as *Cladophora diffusa* is *C. hutchinsiae* Kütz. var. *distans* (Ag.) Kütz., which differs from *Conferva diffusa* Roth (= *Cladophora diffusa* (Roth) Harv.).

(17) Possibly forms of *Cladophora hamosa* Kütz.

(18) Should possibly be *Cladophora crystallina* (Roth) Kütz., see Levring (1937, 1940), Waern, 1952.

(19) = *Conferva tortuosa* Dillw., *Conferva implexa* Harv., *Phyc. Brit.*, 54B, *Lola implexa* (Harv.) Hamel (Chapman, 1939).

(20) Halicystis Areschoug, 1850; gametophyte generation of *Derbesia marina* (Lyngb.) Kjellm. (*Halicystis ovalis* (Lyngb.) Aresch.) recorded for Britain but not the gametophyte generation (*H. parvula* Schmitz) of *Derbesia tenuissima* (De Not.) Crn.

XANTHOPHYCEAE

HETEROCHLORIDALES

Heterochloridaceae
Chloromeson Pascher, 1930.
parvum Carter
Nephrochloris Geitler et Gemisi, 1925.
? *salina* Carter (1)
Olisthodiscus Carter, 1937. (1)
luteus Carter
Rhizochloris Pascher, 1917.
arachnoides Carter

HETEROCOCCALES

Halosphaeraceae
Halosphaera Schmitz, 1879.
viridis Schm.

HETEROTRICHALES

Tribonemataceae
Tribonema Derbès et Solier, 1856.
bombycinum Derb. et Sol.
endozooticum (Wille) Magne
(*Rhizoclonium kerneri* Stockm.
f. *endozooticum* Wille)

HETEROSIPHONALES

Vaucheriaceae (2)
Vaucheria De Candolle, 1801. (3)
compacta (Collins) Collins s.lat.
(*sphaerospora* Nordst. var. *dioica*
K. Rosenv.)
coronata Nordst.
dichotoma (L.) Ag.
intermedia Nordst.
litorea Hofm. et Ag.
sphaerospora Nordst.
synandra Woron.
thuretii Woron.

Notes on Xanthophyceae

(1) Systematic position uncertain.

(2) Reasons for placing in Xanthophyceae—see Chadefaud (1945, 1951), Smith (1950).

(3) The list of species given has been checked by Mr T. Christensen, who also says that the *Vaucheria* sp. recorded by Carter as possibly *V. woroniniana* Heer. is definitely not this species. See also Christensen (1952) for information on distribution.

CHRYSOPHYCEAE

CHRYSOMONADALES (1)

- **Chromulinaceae**
  - CHROMULINA Cienkowski, 1870.
    - *lunaris* Carter
    - *pleiades* Parke
    - *pusilla* Butcher
- **Cyrtophoraceae**
  - PSEUDOPEDINELLA Carter, 1937.
    - *pyriformis* Carter
- **Isochrysidaceae**
  - DICRATERIA Parke, 1949.
    - *gilva* Parke
    - *inornata* Parke
  - ISOCHRYSIS Parke, 1949.
    - *galbana* Parke
- **Ochromonadaceae**
  - OCHROMONAS Wystozki, 1887.
    - *oblonga* Carter
  - PAVLOVA Butcher, 1952.
    - *gyrans* Butcher
- **Prymnesiaceae**
  - PRYMNESIUM Massart, 1920.
    - *minutum* Carter
    - *parvum* Carter

RHIZOCHRYSIDALES

- **Platychrysidaceae**
  - PLATYCHRYSIS Geitler, 1930.
    - *pigra* Geitler

CHRYSOCAPSALES

- **Chrysocapsaceae**
  - CHRYSOTILA Anand, 1937. (2)
    - *lamellosa* Anand
    - *stipitata* Anand
  - GLOEOCHRYSIS Pascher, 1925.
    - *litoralis* Anand
    - *maritima* Anand
  - PHAEOCYSTIS Lagerheim, 1893.
    - *globosa* Scherff.
    - *pouchetii* (Hariot) Lagerh.

CHRYSOTRICHALES

- **Nematochrysidaceae**
  - NEMATOCHRYSIS Pascher, 1925.
    - *sessilis* Pascher
      - var. *vectensis* Carter
- **Phaeothamnionaceae**
  - APISTONEMA Pascher, 1925. (3)
    - *carteri* Anand (4)
  - CHRYSONEMA Anand, 1937.
    - *litorale* Anand
- **Thallochrysidaceae**
  - THALLOCHRYSIS Conrad, 1920.
    - *litoralis* Anand

Notes on Chrysophyceae

(1) Coccolithophoridaceae not included in this preliminary list.

(2) Systematic position uncertain: should possibly be placed as the type of a new family.

(3) *Phaeococcus adnatus* G. S. West ex Cotton (1912), imperfectly described by Cooke and Rabenhorst under the name *Gloeocystis adnata* Näg., belongs, very possibly, to the genus *Apistonema* Pascher.

(4) See Waern (1952)—possibly a form of *A. pyrenigerum* Pascher.

PHAEOPHYCEAE

ECTOCARPALES

- **Ectocarpaceae** (1)
  - ACINETOSPORA Bornet, 1891.
    - *crinita* (Carm.) Kornmann (2)
      - (*pusilla* (Griff.) Born.)
      - (*pusilla* var. *crinita* (Carm.) Batt.)
    - haploid generation =
      - ? *Ectocarpus lebelii* Crn.
      - ? *Ectocarpus padinae* Sauv.
  - CHILIONEMA Sauvageau, 1897. (3)
    - *ocellatum* (Kütz.) Kuck.
      - (*nathaliae* Sauv.)
      - (*ocellatum* (Rke.) Sauv.)
    - *reptans* (Crn.) Sauv.
  - COMPSONEMA Kuckuck, 1899. (3)
    - *microspongium* (Batt.) Kuck.
      - (*Ectocarpus microspongium* Batt.)
    - *saxicola* Kuck.
      - (*Myrionema saxicolum* Kuck.)

- ? DICHOSPORANGIUM Hauck, 1885.
  - ? *chordariae* Wollny (4)
    - (*Streblonema chordariae* Cotton in Newton, p. 128, non *S. chordariae* (Farl.) De Toni)
- ECTOCARPUS Lyngbye, 1819.
  - ? *acanthophorus* Kütz.
  - *confervoides* (Roth) Le Jol. s. lat.
    - (*confervoides* s.str.)
    - (*amphibius* Harv.)
    - (*arctus* Kütz.)
    - (*dasycarpus* Kuck.)
    - (*hiemalis* Crn.)
    - (*penicillatus* (Ag.) Kjellm.)
    - (*siliculosus* (Dillw.) Lyngb.)
  - *congestus* Crn.
  - *crouanii* Thur.
  - ? *distortus* Carm.
  - *draparnaldioides* (Crn.) Kjellm.
  - ? *erectus* Kütz.
  - *fasciculatus* Harv.
  - ? *fenestratus* Berk. ex Harv.
  - *holmesii* Batt.
  - ? *landsburgii* Harv.
  - ? *minimus* Näg. (5)
  - *reinboldii* Rke.
- ENDODICTYON Gran, 1897.
  - *infestans* Gran (9)
    - (*Streblonema infestans* Batt.)
- FELDMANNIA Hamel, 1939.
  - *battersii* (Born.) Hamel
    - (*Ectocarpus battersii* Born.)
  - *globifera* (Kütz.) Hamel
    - (*Ectocarpus globifer* Kütz.) (6)
  - ? *irregularis* (Kütz.) Hamel
    - (*Ectocarpus irregularis* Kütz.)
  - *simplex* (Crn.) Hamel
    - (*Ectocarpus simplex* Crn.)
- GIFFORDIA Batters emend. Hamel, 1939.
  - *granulosa* (Sm.) Hamel
    - (*Ectocarpus granulosus* (Sm.) Ag.)
  - *hincksiae* (Harv.) Hamel
    - (*Ectocarpus hincksiae* Harv.)
  - *mitchellae* (Harv.) Hamel
    - (*Ectocarpus mitchellae* Harv.)
  - *ovata* (Kjellm.) Kylin
    - (*Ectocarpus ovatus* Kjellm.)
    - var. *ovata*
    - var. *arachnoidea* (Rke.) Kylin
  - *sandriana* (Zanard.) Hamel
    - (*Ectocarpus sandrianus* Zanard.)
  - *secunda* (Kütz.) Batt.
    - (*Ectocarpus secundus* Kütz.)
- HECATONEMA Sauvageau 1897. (3)
  - *liechtensternii* (Hauck) Batt.
  - *maculans* (Coll.) Sauv.
  - *speciosum* (Børg.) Cotton
- HERPONEMA J. Agardh emend. Hamel, 1939.
  - *luteolum* (Sauv.) Hamel
    - (*Ectocarpus luteolus* Sauv.)
  - *solitarium* (Sauv.) Hamel
    - (*Ectocarpus solitarius* Sauv.)
  - *valiantei* (Born.) Hamel
    - (*Ectocarpus valiantei* Born.)
  - *velutinum* (Grev.) J. Ag.
    - (*Ectocarpus velutinus* (Grev.) Kütz.) (7)
- LAMINARIOCOLAX Kylin, 1947.
  - *tomentosoides* (Farl.) Kylin
    - (*Ectocarpus tomentosoides* Farl.) (8)
- MIKROSYPHAR Kuckuck, 1895. (9)
  - *polysiphoniae* Kuck.
  - *porphyrae* Kuck.
- PHAEOSTROMA Kuckuck, 1895.
  - *pustulosum* Kuck. (10)
    - (*P. prostratum* Kuck.)
- PYLAIELLA Bory, 1823.
  - *littoralis* (L.) Kjellm. (11)
- SOROCARPUS Pringsheim, 1862.
  - *micromorus* (Bory) Silva
    - (*uvaeformis* (Lyngb.) Pringsh.)
- SPONGONEMA Kützing, 1849.
  - *tomentosum* (Huds.) Kütz.
    - (*Ectocarpus tomentosus* (Huds.) Lyngb. *E. terminalis* Kutz.) (3)
- STREBLONEMA Derbès et Solier, 1851. (12)
  - ? *aequale* Oltm. (10)
  - *breve* (Sauv.) De Toni
    - (*Ectocarpus brevis* Sauv.)
  - *effusum* Kylin
  - *fasciculatum* Thur.
    - var. *fasciculatum*
    - var. ? *simplex* Batt.
  - ? *helophorum* (K. Rosenv.) Batt.
  - *intestinum* (Reinsch) Holm. et Batt.
  - *parasiticum* (Sauv.) De Toni
    - (*Ectocarpus parasiticus* Sauv.)
  - *sphaericum* (Derb. et Sol.) Thur.
  - *stilophorae* (Crn.) De Toni
    - (*Ectocarpus stilophorae* Crn.) (13)
    - var. *stilophorae*
    - var. *caespitosum* (K. Rosenv.) De Toni
  - ? *tenuissimum* Hauck (13)
  - *volubile* (Crn.) Thur.
  - *zanardinii* (Crn.) De Toni
- WAERNIELLA Kylin, 1947.
  - *lucifuga* (Kuck.) Kylin

Lithodermataceae

- LITHODERMA Areschoug, 1875.
  - *extensum* (Crn.) Hamel (14)
    - (*fatiscens* Aresch. emend. Kuck.)
- PETRODERMA Kuckuck, 1897.
  - *maculiforme* (Wollny) Kuck.
- SORAPION Kuckuck, 1894.
  - *simulans* Kuck.

Ralfsiaceae

- RALFSIA Berkeley, 1831. (15)
  - *clavata* (Carm.) Farlow
  - *disciformis* Crn.
  - *pusilla* (Strömf.) Fosl.
  - *spongiocarpa* Batt. (16)
  - *verrucosa* (Aresch.) J. Ag.

Myrionemataceae
  Ascocyclus Magnus, 1874.
    foecundus (Strömf.) Cotton
    hispanicus Sauv.
    magni Sauv.
      (*orbicularis* Magn. in Newton, p. 159)
  Microspongium Reinke, 1888.
    globosum Rke.
      (*Hecatonema globosum* (Rke.) Batt.)
  Myrionema Greville, 1827.
    aecidioides (K. Rosenv.) Sauv. (12)
    corunnae Sauv.
    papillosum Sauv.
    polycladum Sauv.
    strangulans Grev.
  Symphyocarpus Kolderup Rosenvinge, 1894.
    strangulans K. Rosenv.
  ? Ulonema Foslie, 1894. (17)
    ? *rhizophorum* Fosl.

Elachistaceae
  Elachista Duby, 1830. (18)
    flaccida (Dillw.) Aresch.
    fucicola (Vell.) Aresch.
      f. *fucicola*
      f. *grevillei* (Arnott) Hamel
        (*E. grevillei* Arnott)
    scutulata (Sm.) Duby
  Halothrix Reinke, 1888.
    lumbricalis (Kütz.) Rke.
  Leptonema Reinke, 1888.
    fasciculatum Rke.
      var. *fasciculatum*
      var. ? *majus* Rke. (19)
      var. ? *subcylindrica* K. Rosenv. (19)
      var. ? *uncinatum* Rke. (19)
  Symphoricoccus Reinke, 1888.
    stellaris (Aresch.) Kuck. (20)
      (*Elachista stellaris* Aresch.)

Leathesiaceae
  Corynophlaea Kützing, 1843.
    crispa (Harv.) Kuck.
      (*Leathesia crispa* Harv.)
  Cylindrocarpus Crouan, 1851.
      (*Petrospongium* Näg.)
    berkeleyi (Grev.) Crn.
      (*Petrospongium berkeleyi* Näg.)
    microscopicus Crn.
      (*Ectocarpus microscopicus* Batt.)
  Leathesia S. F. Gray, 1821.
    difformis (L.) Aresch.
  Microcoryne Strömfelt, 1888.
    ocellata Strömf. (21)
  Myriactula Kuntze, 1898.
      (*Myriactis* Kütz.)
    areschougii (Crn.) Hamel
    chordae (Aresch.) Levring
      (*Elachista stellaris* var. *chordae* Aresch.)
    clandestina (Crn.) J. Feldm.
      (*Ectocarpus clandestinus* (Crn.) Sauv.)
    haydenii (Gatty) Levring
    rivulariae (Suhr) J. Feldm.
      (*Myriactis pulvinata* Kütz.)
    stellulata (Griff.) Levring

Mesogloiaceae
  Chordaria Agardh emend. Lyngbye, 1819.
    flagelliformis (Müll.) Ag.
      var. *flagelliformis*
      var. ? *firmus* Kjellm.
      var. ? *minor* J. Ag.
  Cladosiphon Kützing, 1843.
    contortus (Thur.) Kylin
      (*Castagnea contorta* Thur.)
    zosterae (J. Ag.) Kylin
      (*Castagnea zosterae* (J. Ag.) Thur.)
  Eudesme J. Agardh, 1880.
    virescens (Carm.) J. Ag.
      (*Castagnea virescens* (Carm.) Thur.)
  Liebmannia J. Agardh, 1842.
    leveillei J. Ag.
      (*Mesogloia leveillei* Menegh.)
  Mesogloia Agardh, 1817.
    lanosa Crn.
    ? *neglecta* Batt. (22)
    vermiculata (Sm.) Le Jol.
  Myriocladia J. Agardh, 1841. (23)
    tomentosa Crn.
  Sauvageaugloia Hamel, 1939.
    griffithsiana (Grev.) Hamel
      (*Mesogloia griffithsiana* Grev.)
  Sphaerotrichia Kylin, 1940.
    divaricata (Ag.) Kylin
      (*Chordaria divaricata* Ag.)
  Strepsithalia Sauvageau, 1896.
    buffhamiana (Batt.) Batt.

Acrothricaceae
  Acrothrix Kylin, 1907.
    gracilis Kylin

Spermatochnaceae
  Spermatochnus Kutzing emend. Reinke, 1889.
    paradoxus (Roth) Kütz.
  Stilophora J. Agardh, 1841.
    rhizodes (Ehrh.) J. Ag.
    tuberculosa (Horn.) Rke.
  Stilopsis Kuckuck, 1929.
    lejolisii (Thur.) Kuck.
      (*Spermatochnus lejolisii* Rke.)

DICTYOSIPHONALES

Striariaceae
  Isthmoplea Kjellman, 1877.
    sphaerophora (Carm.) Kjellm.
  Stictyosiphon Kützing, 1843.
    griffithsianus (Le Jol.) Holm. et Batt. (24)
      (*Phloeospora brachiata* Born. in Newton, p. 187)
    soriferus (Rke.) K. Rosenv. (25)
    subarticulatus (Aresch.) Hauck (25)
    tortilis (Rupr.) Rke. (25)
  Striaria Greville, 1829.
    attenuata Grev.
      f. *attenuata*
      f. *crinita* (Ruch.) Hauck

Myriotrichiaceae
Buffhamia Batters, 1895. (26)
speciosa Batt.
Leblondiella Hamel, 1939.
densa (Batt.) Hamel
(*Myriotrichia densa* Batt.)
Myriotrichia Harvey, 1834.
clavaeformis Harv.
var. *clavaeformis*
var. ? *minima* Holm. et Batt.
? *filiformis* Harv. (27)
repens (Hauck) Karsakoff

Giraudiaceae
Giraudia Derbès et Solier, 1851. (26)
sphacelarioides Derb. et Sol.

Punctariaceae
Desmotrichum Kützing, 1845.
undulatum (J. Ag.) Rke.
(*balticum* Kütz.) (28)
(*Punctaria tenuissima* Grev.)
Litosiphon Harvey, 1849.
filiformis (Rke.) Batt.
var. *filiformis*
var. ? *gracilis* Batt.
laminariae (Lyngb.) Harv.
(*hibernicus* (Johns.) Batt.)
pusillus (Carm.) Harv.
Punctaria Greville, 1830. (29)
crispata (Kütz.) Batt.
latifolia Grev.
var. *latifolia*
var. ? *laminarioides* Holm. et Batt.
var. ? *lanceolata* Batt.
plantaginea Grev.
var. *plantaginea*
var. ? *crouanii* Thur.
var. ? *rubescens* Batt.

Asperococcaceae
Asperococcus Lamouroux, 1813.
bullosus Lamour.
compressus Griff. ex Hook. (30)
fistulosus (Huds.) Hook.
f. *fistulosus*
f. *vermicularis* (Griff.) Harv.
scaber Kuck.

Dictyosiphonaceae
Dictyosiphon Greville, 1830. (31)
chordaria Aresch.
(*mesogloia* Aresch.)
(*Gobia baltica* Rke.)
ekmanii Aresch.
foeniculaceus (Huds.) Grev.
f. *foeniculaceus*
f. *hippuroides* (Lyngb.) Levring
(*D. hippuroides* Kütz.)

SCYTOSIPHONALES (32)

Phaeosaccionaceae
Phaeosaccion Farlow, 1882.
collinsii Farl.

Scytosiphonaceae
Colpomenia Derbès et Solier, 1856.
peregrina Sauv.
Petalonia Derbès et Solier, 1850. (33)
fascia (Müll.) Kuntze (34)
(*debilis* (Ag.) Derb. et Sol.)
(*Phyllitis fascia* Kütz.)
? *filiformis* (Batt.)
zosterifolia (Rke.) Kuntze
(*Phyllitis zosterifolia* Rke.)
Scytosiphon Agardh, 1811.
lomentaria (Lyngb.) Endl.
(*pygmaeus* Rke.)
var. *lomentaria*
var. ? *zostericola* Thur.

TILOPTERIDALES

Tilopteridaceae
Haplospora Kjellman, 1872.
globosa Kjellm.
Tilopteris Kützing, 1849.
mertensii (Sm.) Kütz.

CUTLERIALES

Cutleriaceae
Cutleria Greville, 1830.
multifida (Sm.) Grev.
sporophyte = *Aglaozonia parvula* (Grev.) Zanard. (*A. reptans* Crn.)
Zanardinia Nardo, 1841.
prototypus Nardo
(*collaris* Crn.)

SPOROCHNALES

Sporochnaceae
Carpomitra Kützing, 1843.
costata (Stackh.) Batt.
Sporochnus Agardh, 1817.
pedunculatus (Huds.) Ag.

DESMARESTIALES

Arthrocladiaceae
Arthrocladia Duby, 1832.
villosa (Huds.) Duby

Desmarestiaceae
Desmarestia Lamouroux, 1813.
aculeata (L.) Lamour.
dudresnayi Lamour.
ligulata (Lightf.) Lamour.
var. *ligulata*
var. ? *angustior* Batt.
var. ? *dilatata* Batt.
viridis (Müll.) Lamour.

LAMINARIALES

Chordaceae
- CHORDA Stackhouse, 1797. (35)
  - *filum* (L.) Stackh.
  - *tomentosa* Lyngb.

Laminariaceae
- LAMINARIA Lamouroux, 1813.
  - *digitata* (Huds.) Lamour.
  - *hyperborea* (Gunn.) Fosl.
    - (*cloustonii* Edm.)
  - *ochroleuca* La Pylaie
  - *saccharina* (L.) Lamour.
    - (*hieroglyphica* J. Ag.)
- SACCORHIZA La Pylaie, 1829.
  - *polyschides* (Lightf.) Batt.
    - (*bulbosa* (Huds.) La Pylaie)

Alariaceae
- ALARIA Greville, 1830.
  - *esculenta* (L.) Grev.

SPHACELARIALES

Choristocarpaceae
- CHORISTOCARPUS Zanardini, 1860.
  - *tenellus* (Kütz.) Zanard.

Sphacelariaceae
- BATTERSIA Reinke, 1890.
  - *mirabilis* Rke.
- CHAETOPTERIS Kützing, 1843.
  - *plumosa* (Lyngb.) Kütz.
- SPHACELARIA Lyngbye, 1819. (36)
  - *bipinnata* (Kütz.) Sauv.
  - *britannica* Sauv.
  - *caespitula* Lyngb.
  - *cirrosa* (Roth) Ag.
  - *fusca* (Huds.) Ag.
  - *plumigera* Holm.
  - *plumula* Zanard.
  - *racemosa* Grev.
  - *radicans* (Dillw.) Ag.
  - ? *saxatilis* (Kuck.) Sauv.
  - *tribuloides* Menegh.
- SPHACELLA Reinke, 1890.
  - *subtilissima* Rke.

Stypocaulaceae
- HALOPTERIS Kützing emend. Sauvageau, 1903.
  - *filicina* (Grat.) Kütz.
    - var. *filicina*
    - var. *patentissima* Sauv.
      - (*sertularia* Batt.)
  - *scoparia* (L.) Sauv.
    - var. *scoparia*
    - var. *patentissima* Sauv.
      - (*scoparioides* Holm. et Batt.)

Cladostephaceae
- CLADOSTEPHUS Agardh, 1817.
  - *spongiosus* (Huds.) Ag.
  - *verticillatus* (Lightf.) Ag.

DICTYOTALES

Dictyotaceae
- DICTYOPTERIS Lamouroux, 1809.
  - *membranacea* (Stackh.) Batt.
- DICTYOTA Lamouroux, 1809.
  - *dichotoma* (Huds.) Lamour.
    - var. *dichotoma*
    - var. *intricata* (Ag.) Grev.
    - var. ? *latifrons* Holm. et Batt.
- ILOPHUS J. Agardh, 1880.
  - *spiralis* (Mont.) Hamel
    - (*Dictyota ligulata* Kütz.)
- PADINA Adanson, 1763.
  - *pavonia* (L.) Lamour.
- TAONIA J. Agardh, 1848.
  - *atomaria* (Woodw.) J. Ag.
    - var. *atomaria*
    - var. ? *divaricata* Holm. et Batt.

FUCALES

Fucaceae
- ASCOPHYLLUM Stackhouse, 1809.
  - *nodosum* (L.) Le Jol.
    - f. *nodosum*
    - f. *mackaii* (Turn.)
      - (*A. mackaii* (Turn.) Holm. et Batt.)
    - f. *scorpioides* (Hornem.) Hauck
- FUCUS Linnaeus, 1753. (37)
  - *ceranoides* L.
  - *inflatus* L.
    - (*anceps* Harv. et Ward.)
  - *serratus* L.
  - *spiralis* L.
  - *vesiculosus* L.
- PELVETIA Decaisne et Thuret, 1845.
  - *canaliculata* (L.) Dcne. et Thur.

Himanthaliaceae
- HIMANTHALIA Lyngbye, 1819.
  - *elongata* (L.) S. F. Gray
    - (*lorea* (L.) Lyngb.)

Cystoseiraceae
- BIFURCARIA Stackhouse, 1809.
  - *rotunda* (Huds.) Papenf.
    - (*tuberculata* (Huds.) Stackh.)
- CYSTOSEIRA Agardh, 1820.
  - *fibrosa* (Huds.) Ag.
  - *foeniculacea* (L.) Grev. emend. Sauv.
    - (*discors* Ag.)
  - *granulata* Ag.
  - *tamariscifolia* (Huds.) Papenf.
    - (*ericoides* (L.) Ag.)
- HALIDRYS Lyngbye, 1819.
  - *siliquosa* (L.) Lyngb.

Notes on Phaeophyceae

(1) British material belonging to genera in this family is in urgent need of revision.

(2) See Kornmann (1953): Dr Kornmann very kindly sent me proofs of his paper and also of that of Kuckuck (1953), so that the information contained in these papers could be included here.

(3) See Kuckuck (1953).

(4) The genus *Dichosporangium* is included here for the present to cover *Streblonema chordariae* Cotton in Newton (1931). If remaining in *Streblonema* it would need a new name because of earlier *S. chordariae* (Farl.) De Toni.

(5) Requires investigation. Hamel (1939), suggests it should be placed in *Herponema* J. Ag. emend. Hamel.

(6) New combination not made for var. *rupestris* Batt., investigation needed.

(7) New combination not made for var. *laterifructus* Batt., investigation needed.

(8) New combination not made for var. *punctiformis* Batt., investigation needed.

(9) See Levring (1945) and Waern (1952).

(10) Jaasund (1951) considers genus should be placed in the Chordariaceae; also considers that *Streblonema aequale* Oltm. is possibly a form of *Phaestroma pustulosum*. According to Cotton (1912) British material differs slightly from Kuckuck's plant and may possibly be a distinct species.

(11) Concerning validity of name and also *Pylaiella rupincola* (Aresch.) Kylin—see information given in Waern (1952).

(12) *Streblonema* s.lat.; British material belonging to this complex in urgent need of investigation and revision, see Hamel (1939), Kylin (1947), Waern (1952). British species placed by Hamel (1939) in *Entonema* Reinsch sensu Hamel (*Streblonema parasiticum* (Sauv.) De Toni, *S. breve* (Sauv.) De Toni, *Ectocarpus clandestinus* (Crn.) Sauv.) and by Kylin (1947) in *Entonema* Reinsch sensu Kylin (*Streblonema effusum* Kylin, *S. aequale* Oltm., *Myrionema aecidioides* (K. Rosenv.) Sauv.) have, for the time being, been left, either in the genus *Streblonema* s.lat., or in other genera in which they have been placed.

(13) *Ectocarpus stilophorae* Crn. var. *cervicornis* Batt. recorded, investigation needed. Is *Streblonema tenuissimum* Hauck a form of *S. stilophorae* (Crn.) De Toni?

(14) Almost certainly more than one species recorded for British coast under name *L. fatiscens* Aresch., see Waern (1952). British material of whole family in need of investigation.

(15) Genus in need of revision, see Hamel (1939), Hollenberg (1941), Kylin (1947).

(16) According to Batters = *R. clavata* Rke. *non* Farlow; see Kylin (1947) who gives new name *R. tenuis* for Reinke's *R. clavata*; requires investigation.

(17) See Cotton (1912), Levring (1937), Kylin (1947), Jaasund (1951).

(18) Conserved as *Elachista*, original spelling *Elachistea*, which is etymologically incorrect according to Fritsch (1945).

(19) Habitat forms of species?

(20) = *Areschougia stellaris* (Aresch.) Menegh. See Silva (1952), where *Areschougia* Harv. is proposed for conservation against *Areschougia* Menegh.

(21) See Hamel (1939).

(22) See Batters (1906), and Cotton (1912, p. 124, under *Acrothrix*.)

(23) According to Kylin (1933) the plant recorded on the British coast under the name *Myriocladia lovenii* is not the true *M. lovenii* J. Ag.; it may be an undescribed species.

(24) The following notes are from Mr R. Ross and Miss L. Newton concerning the confusion that has been found in the synonomy dealing with this plant. The type specimen of *Conferva brachiata* Sm. in Engl. Bot. has been examined and is thought to be a *Pylaiella*. This specimen is also the nomenclatural type of *Ectocarpus brachiatus* (Sm.) Harv. *ex* Hook. *non* C. Ag., *Phloeospora brachiata* (Sm.) Bornet, *Stictyosiphon brachiatus* (Sm.) Hygen et Jorde. The plant found by Mrs Griffiths and described and figured under the above names by all authors is quite different. Its earliest legitimate name is *Ectocarpus griffithsianus* Le Jolis in *Trans. bot. Soc. Edinb.*, vol. 7, p. 37 (1863), and a combination based on this name in the appropriate genus is its correct name. The plant has been left for the time being in the genus *Stictyosiphon*, but further study is needed.

(25) British material in urgent need of investigation; kept as three separate species for the present, but see Kolderup Rosenvinge (1935), Levring (1937, 1940), Kolderup Rosenvinge & Lund (1947), Waern (1952).

(26) Systematic position uncertain.

(27) British material needs investigation; should be perhaps *M. clavaeformis* f. *filiformis* (Harv.) Kjellm., see Kolderup Rosenvinge & Lund (1947), Kylin (1947).

(28) See Kylin (1947), Kolderup Rosenvinge & Lund (1947).

(29) British material needs study, see Hamel (1939), Kolderup Rosenvinge & Lund (1947).

(30) See Hamel (1939), who places this species in genus *Haloglossum* Kütz.

(31) British material of genus in need of investigation; see Du Rietz (1940), Levring (1940), Kolderup Rosenvinge & Lund (1947), Sinclair (1949).

(32) See J. Feldmann (1949).

(33) See Silva (1952) concerning name of genus.

(34) See Setchell & Gardner (1925) under *Ilea* Fries; field observations needed before British forms of species can be revised and the limits of the species assessed.

(35) Recorded varieties of both species require investigation before being listed. Kolderup Rosenvinge & Lund (1947) state that the var. *subtomentosa* of *Chorda filum* is not to be maintained.

(36) British material of genus in need of revision, see Waern (1945, 1952), Kylin (1947), Lund (1950).

(37) No varieties or forms given in list; awaiting the results of Dr E. Burrows and Dr S. Lodge's work on the form-range of the *Fucus* species (except *F. inflatus*) on the British coast. Mr H. Powell is investigating *F. inflatus* L.

RHODOPHYCEAE

BANGIOPHYCIDAE

GONIOTRICHALES

- **Goniotrichaceae**
  - Asterocytis Gobi, 1879.
    - *ramosa* (Thwaites) Gobi (1)
  - Goniotrichum Kützing, 1843.
    - ? *elegans* (Chauv.) Le Jol.
    - *cornu-cervi* (Reinsch) Hauck
  - Neevea Batters, 1900.
    - *repens* Batt.

BANGIALES

- **Erythropeltidaceae**
  - Erythrocladia Kolderup Rosenvinge, 1909.
    - *irregularis* K. Rosenv.
    - *subintegra* K. Rosenv.
  - Erythropeltis Schmitz, 1896. (2)
    - ? *discigera* Schm.
      - var. ? *discigera*
      - var. ? *flustrae* Batt.
  - Erythrotrichia Areschoug, 1850. (3)
    - *bertholdii* Batt.
    - *boryana* (Mont.) Berth.
      - var. *boryana*
      - var. ? *crispa* Batt.
    - *carnea* (Dillw.) J. Ag.
    - *ciliaris* (Carm.) Batt.
    - *investiens* (Zanard.) Born.
    - *reflexa* (Crn.) Thur.
    - *welwitschii* (Rupr.) Batt.
  - Porphyropsis Kolderup Rosenvinge, 1909.
    - *coccinea* (J. Ag.) K. Rosenv.
- **Bangiaceae**
  - Bangia Lyngbye, 1819.
    - *fuscopurpurea* (Dillw.) Lyngb. (4)
  - Conchocelis Batters, 1892. (5)
    - ? *rosea* Batt.
  - Porphyra Agardh, 1824.
    - *amethystea* Kütz.
    - *leucosticta* Thur.
    - *linearis* Grev.
    - *miniata* (Lyngb.) Ag.
      - f. *miniata*
      - f. *abyssicola* (Kjellm.) K. Rosenv.
      - f. *amplissima* (Kjellm.) K. Rosenv.
      - f. *tenuissima* (Strömf.) K. Rosenv.
    - *umbilicalis* (L.) Kütz.
      - f. *umbilicalis*
      - f. *laciniata* (Lightf.) J. Ag.

FLORIDEOPHYCIDAE

NEMALIONALES

- **Acrochaetiaceae**
  - Acrochaetium Nägeli, 1861. (6)
    - *alariae* (Jonss.) Born.
    - *battersianum* Hamel
    - *bonnemaisoniae* (Batt.) J. et G. Feldm.
      - (*Colaconema bonnemaisoniae* Batt.)
    - *bornetii* Papenf.
      - (*corymbiferum* (Thur.) Batt.)
    - *brebneri* (Batt.) Hamel
      - (*Rhodochorton brebneri* Batt.)
    - *caespitosum* (J. Ag.) Näg.
      - ? (*lorrain-smithiae* (Lyle) Newton)
    - *chylocladiae* (Batt.) Batt.
      - f. *chylocladiae*
      - f. *pulchrum* Batt.
    - *daviesii* (Dillw.) Näg.

Acrochaetium (*cont.*)
emergens (K. Rosenv.) Web.-v. Bosse
endophyticum (Batt.) Batt.
endozoicum (Darb.) Batt.
microscopicum (Näg.) Näg.
minutum (Suhr) Hamel
(*Rhodochorton minutum* (Suhr) Rke.)
mirabile Näg.
nemalionalis (De Not.) Born.
pallens (Zanard.) Näg.
(*Rhodochorton pallens* (Zanard.) Hauck)
parvulum (Kylin) Hoyt
reticulatum (Batt.) Papenf.
(*Colaconema reticulatum* Batt.)
sanctae-mariae (Darb.) Hamel
scapae (Lyle) Papenf.
secundatum (Lyngb.) Näg.
seiriolanum (Harvey-Gibs.) K. Rosenv.
sparsum (Harv.) Näg.
thuretii (Born.) Collins et Hervey
trifilum (Buff.) Batt.
virgatulum (Harv.) J. Ag.
f. *virgatulum*
f. *luxurians* (J. Ag.)
f. *tetrica* (K. Rosenv.)
? *griffithsianum* Näg.
? *irregulare* Reinsch
? *lanuginosum* (Dillw.) Näg.
? *pulvereum* Näg.
Audouinella Bory emend. Papenfuss, 1945.
efflorescens (J. Ag.) Papenf.
(*Acrochaetium efflorescens* Näg.)
membranacea (Magn.) Papenf.
(*Rhodochorton membranaceum* Magn.)
Rhodochorton Nägeli, 1861. (7)

Helminthocladiaceae
Helminthocladia J. Agardh, 1851.
calvadosii (Lamour.) Setch.
(*purpurea* J. Ag.)
? *hudsonii* J. Ag.
Helminthora J. Agardh, 1851.
divaricata (Ag.) J. Ag.
Nemalion Duby, 1830.
elminthoides (Vell.) Batt.
? *multifidum* (Web. et Mohr) J. Ag.

Chaetangiaceae
Scinaia Bivona, 1822.
furcellata (Turn.) Bivona
subcostata (J. Ag.) Chemin ex Hamel
(*furcellata* var. *subcostata* J. Ag.

GELIDIALES

Gelidiaceae
Gelidium Lamouroux, 1813. (8)
Pterocladia J. Agardh, 1852.
pinnata (Huds.) Papenf.
(*capillacea* (Gmel.) Born. et Thur.)

GIGARTINALES

Cruoriaceae
Cruoria Fries, 1835.
pellita (Lyngb.) Fries
rosea Crn.
var. *rosea*
var. *purpurea* (Crn.) Batt.
Cruoriopsis Dufour, 1864.
gracilis (Kuck.) Batt.
? *hauckii* Batt. (9)
Petrocelis J. Agardh, 1852.
cruenta J. Ag.
hennedyi (Harv.) Batt.

Calosiphoniaceae
Calosiphonia Crouan, 1852.
vermicularis (J. Ag.) Schm.

Nemastomaceae (10)
Platoma Schmitz, 1889.
bairdii (Farl.) Kuck.
marginifera J. Ag.
Schizymenia J. Agardh, 1851.
dubyi (Chauv.) J. Ag.

Furcellariaceae
Furcellaria Lamouroux, 1813.
fastigiata (L.) Lamour. (11)
Halarachnion Kützing, 1843.
ligulatum (Woodw.) Kütz.

Rhabdoniaceae
Catenella Greville, 1830.
repens (Lightf.) Batt.

Rhodophyllidaceae
Calliblepharis Kützing, 1843.
ciliata (Huds.) Kütz.
var. *ciliata*
var. ? *angusta* Holm. et Batt.
lanceolata (Stackh.) Batt.
Cystoclonium Kützing, 1843.
purpureum (Huds.) Batt.
Rhodophyllis Kützing, 1847.
? *appendiculata* J. Ag.
divaricata (Stackh.) Papenf. (12)
(*bifida* Kütz.)

Plocamiaceae
Plocamium Lamouroux, 1813.
coccineum (Huds.) Lyngb.
f. *coccineum*
f. *uncinatum* (Ag.) Levring

Sphaerococcaceae
Sphaerococcus Stackhouse, 1797.
coronopifolius Stackh.

Gracilariaceae
Cordylecladia J. Agardh, 1852.
erecta (Grev.) J. Ag.
Gracilaria Greville, 1830.
bursa-pastoris (Gmel.) Silva
(*compressa* (Ag.) Grev.)
foliifera (Forssk.) Børg.
(*multipartita* J. Ag.)
verrucosa (Huds.) Papenf.
(*confervoides* (L.) Grev.)
f. *verrucosa*
f. *gracilis* (Stackh.)
f. *procerrima* (Turn.)

Phyllophoraceae

- Ahnfeltia Fries, 1835.
  - *plicata* (Huds.) Fries
- Gymnogongrus Martius, 1833.
  - *griffithsiae* (Turn.) Mart.
  - *norvegicus* (Gunn.) J. Ag.
  - *patens* J. Ag.
- ? Petroglossum Hollenberg, 1943.
  - *nicaeense* (Duby) Schotter (13)
    - (*Rhodymenia palmetta* var. *elisiae* Chauv.)
- Phyllophora Greville, 1830. (14)
  - *brodiaei* (Turn.) J. Ag.
  - *membranifolia* (Good. et Woodw.) J. Ag.
  - *palmettoides* J. Ag.
  - *rubens* (L.) Grev.
    - (*epiphylla* (Müll.) Batt.)
  - *traillii* Holm. et Batt.
- Stenogramme Harvey, 1841.
  - *interrupta* (Ag.) Mont.

Gigartinaceae

- Chondrus Stackhouse, 1797.
  - *crispus* (L.) Stackh. (15)
- Gigartina Stackhouse, 1809.
  - *acicularis* (Wulf.) Lamour.
  - *pistillata* (Gmel.) Stackh.
  - *stellata* (Stackh.) Batt. (15)
  - *teedii* Lamour.

CRYPTONEMIALES

Gloiosiphoniaceae

- Gloiosiphonia Carmichael ex Berkeley, 1833.
  - *capillaris* (Huds.) Carm.

Dumontiaceae

- Dilsea Stackhouse, 1809.
  - *carnosa* (Schmidel) Kuntze
    - (*edulis* (Stackh.) Stackh.)
- Dudresnaya Crouan frat., 1835.
  - *verticillata* (With.) Le Jol.
- Dumontia Lamouroux, 1813.
  - *incrassata* (Müll.) Lamour.

Polyideaceae

- Polyides Agardh, 1822.
  - *caprinus* (Gunn.) Papenf.
    - (*rotundus* (Gmel.) Grev.)

Squamariaceae (16)

- Erythrodermis Batters, 1900. (17)
  - *allenii* Batt.
- Haematocelis J. Agardh, 1852. (17)
  - *rubens* J. Ag.
- Peyssonelia Decaisne, 1841.
  - *atropurpurea* Crn.
  - *dubyi* Crn.
    - (*Cruoriella dubyi* (Crn.) Schm.)
  - *harveyana* Crn.
  - *rosenvingii* Schm.
  - *rubra* (Grev.) J. Ag.
  - *rupestris* Crn.
- Porphyrodiscus Batters, 1897. (17)
  - *simulans* Batt.
- Rhododermis Crouan, 1852.
  - *elegans* Crn.
    - var. *elegans*
    - var. *polystromatica* Batt. (18)
  - *georgii* (Batt.) Collins
    - (*Rhodophysema georgii* Batt.)
  - ? *parasitica* Batt. (19)
- Rhododiscus Crouan, 1859.
  - *pulcherrimus* Crn.

Hildenbrandiaceae

- Hildenbrandia Nardo, 1834.
  - *crouanii* J. Ag.
  - *prototypus* Nardo

Corallinaceae (20)

- Choreonema Schmitz, 1889.
  - *thuretii* (Born.) Schm.
- Corallina Linnaeus emend. Lamouroux, 1812.
  - ? *elongata* Johnst. (21)
  - *granifera* Ellis et Soland.
    - (*virgata* Zanard.)
  - *officinalis* L.
    - f. *officinalis*
    - f. *compacta* (Crn.)
  - *squamata* Ellis
- Epilithon Heydrich, 1897. (22)
  - ? *membranaceum* (Esper) Heydr.
    - (*Lithothamnion membranaceum* Fosl.)
- Jania Lamouroux, 1812.
  - *corniculata* (L.) Lamour.
    - (*Corallina rubens* var. *corniculata* Hauck)
  - *rubens* (L.) Lamour.
    - (*Corallina rubens* Ellis et Sol.)
- Lithophyllum Philippi, 1837.
  - *adplicitum* (Fosl.) Newton
  - *corallinae* (Crn.) Heydr.
    - (*Melobesia corallinae* Crn.)
  - *crouanii* Fosl.
  - *fasciculatum* Fosl.
    - f. *complanata* Fosl.
    - f. *divergens* Fosl.
    - f. *incrassata* Fosl.
  - *hapalidioides* (Crn.) Heydr.
    - f. *hapalidioides*
    - f. *confinis* (Crn.) Fosl.
  - *incrustans* Phil.
    - f. *incrustans*
    - f. *depressa* (Crn.) Fosl.
    - f. *harveyi* Fosl.
    - f. *subdichotomum* Heydr.
  - *orbiculatum* Fosl. (23)
  - *pustulatum* (Lamour.) Fosl.
    - f. *pustulatum*
    - f. *laminariae* (Crn.) Fosl.
- Lithothamnion Philippi, 1837.
  - *bornetii* Fosl.
  - *calcareum* (Pall.) Aresch.
    - f. *calcareum*
    - f. *compressa* (McCalla) Fosl.
    - f. *crassa* Lem.
    - f. *squarrulosa* Fosl.

Lithothamnion (*cont.*)
calcareum (*cont.*)
f. *subsimplex* (Batt.) Fosl.
f. ? *subvalida* Fosl.
? *colliculosum* Fosl. (24)
f. *colliculosum*
f. *rosea* Batt.
compactum Kjellm.
? *fruticulosum* (Kütz.) Fosl.
glaciale Kjellm.
granii Fosl. (24)
f. *reducta* Fosl. (*battersii* Fosl.)
laeve (Strömf.) Fosl.
(*stroemfeltii* Fosl.)
laevigatum Fosl.
lenormandii (Aresch.) Fosl.
f. *lenormandii*
f. *squamulosa* Fosl.
f. *sublaevis* Fosl.
norvegicum (Aresch.) Kjellm.
(*calcareum* var. *norvegicum* Fosl.)
polymorphum (L.) Aresch.
sonderi Hauck
f. *sonderi*
f. *sublaevigata* Fosl.
Melobesia Lamouroux, 1812. (22)
? *farinosa* Lamour.
var. *farinosa*
var. *borealis* Lemoine
var. *solmsiana* Fkbg.
? *lejolisii* Rosan.
? *minutula* Fosl.
? *zonalis* (Crn.) Fosl.
Mesophyllum Lemoine, 1928.
lichenoides (Ellis) Lemoine
(*Lithothamnion lichenoides* Fosl.)
f. *lichenoides*
f. *agariciformis* (Johnst. ?)
f. *depressa* (Fosl.)
Schmitziella Bornet et Batters, 1892.
endophlaea Born. et Batt.

Grateloupiaceae
Grateloupia Agardh, 1822.
dichotoma J. Ag.
filicina (Wulf.) Ag.
var. *filicina*
var. *intermedia* Holm. et Batt.
minima Crn.
Halymenia Agardh, 1817.
latifolia Crn.

Kallymeniaceae
Callocolax Schmitz, 1895.
neglectus Schm.
Callophyllis Kützing, 1843.
flabellata Crn.
laciniata (Huds.) Kütz.
Euthora J. Agardh, 1847.
cristata (L.) J. Ag.
Kallymenia J. Agardh, 1842. (25)
? *larterae* Holm.
reniformis (Turn.) J. Ag.
var. *reniformis*
var. *ferrarii* J. Ag.
var. *undulata* J. Ag.
Meredithia J. Agardh, 1892.
microphylla J. Ag.
(*Kallymenia microphylla* J. Ag.)

Choreocolaceae
Choreocolax Reinsch, 1875.
polysiphoniae Reinsch
Harveyella Schmitz et Reinke, 1889.
mirabilis (Reinsch) Schm. et Rke.
Holmsella Sturch, 1926.
pachyderma (Reinsch) Sturch

BONNEMAISONIALES (26)

Naccariaceae
Atractophora Crouan, 1849.
hypnoides Crn.
Naccaria Endlicher, 1836.
wiggii (Turn.) Endl.

Bonnemaisoniaceae
Asparagopsis Montagne, 1840.
armata Harv.
tetrasporophyte = *Falkenbergia rufolanosa* (Harv.) Schm.
Bonnemaisonia Agardh, 1822.
asparagoides (Woodw.) Ag.
tetrasporophyte = *Hymenoclonium serpens* (Crn.) Batt.
clavata (Schousb.) Hamel
hamifera Hariot
tetrasporophyte = *Trailliella intricata* Batt.

RHODYMENIALES

Champiaceae
Champia Desvaux, 1808.
parvula (Ag.) Harv.
Chylocladia Greville, 1833.
reflexa Lenorm.
squarrosa (Kütz.) Le Jol.
(*kaliformis* var. *squarrosa* Harv.)
verticillata (Lightf.) Bliding
(*kaliformis* (Good. et Woodw.) Grev.)
var. *verticillata*
var. *patens* (Kütz.)
Gastroclonium Kützing, 1843.
ovatum (Huds.) Papenf.
(*Chylocladia ovata* Batt.)
var. *ovatum*
var. *subarticulatum* (Kütz.)
Lomentaria Lyngbye, 1819.
articulata (Huds.) Lyngb.
clavellosa (Turn.) Gaill.
var. *clavellosa*
var. *sedifolia* Harv.
orcadensis (Harv.) Collins
(*rosea* (Harv.) Thur.)

Rhodymeniaceae
Rhodymenia Greville, 1830.
ardissonei J. Feldm.
(*corallicola* Ardiss.)
palmata (L.) Grev. (27)
pseudopalmata (Lamour.) Silva (27)
(*palmetta* Grev.)

CERAMIALES

Ceramiaceae

- Aglaothamnion G. Feldmann-Mazoyer, 1940.
  - *brodiaei* (Harv.) G. Feldm.
    - (*Callithamnion brodiaei* Harv.)
  - *polyspermum* (Ag.) Parke
    - (*Callithamnion polyspermum* Ag.)
    - f. *polyspermum*
    - f. *scopulorum* (Ag.) Parke
      - (*Aglaothamnion scopulorum* (Ag.) G. Feldm.)
  - *tenuissimum* (Bonnem.) G. Feldm.
    - (*Callithamnion tenuissimum* (Bonnem.) Kütz.)
  - *tripinnatum* (Grat.) G. Feldm.
    - (*Callithamnion tripinnatum* (Grat.) Ag.)
- Antithamnion Nägeli, 1847.
  - *boreale* (Gobi) Kjellm.
  - *cruciatum* (Ag.) Näg.
    - var. *cruciatum*
    - var. *pumilum* (Harv.) Batt.
  - *floccosum* (Müll.) Kleen
  - *plumula* (Ellis) Thur.
    - var. *plumula*
    - var. *crispum* (Ducluz.) Hauck
    - var. *spinescens* Strömf.
  - *sarniense* (Lyle) G. Feldm. (28)
    - (*Antithamnionella sarniensis* Lyle)
  - *spirographidis* Schiffner
- Bornetia Thuret, 1855.
  - *secundiflora* (J. Ag.) Thur.
- Callithamnion Lyngbye emend. G. Feldmann-Mazoyer, 1940. (29)
  - *arbuscula* (Dillw.) Lyngb.
  - *byssoides* Arnott
  - *corymbosum* (Sm.) Lyngb.
  - *dudresnayi* Crn.
  - ? *fruticulosum* J. Ag. (30)
  - *granulatum* (Ducluz.) Ag. (31)
  - *hookeri* (Dillw.) Ag.
  - *rabenhorstii* Crn.
  - ? *roseum* Harv. (32)
  - ? *spongiosum* Harv. (31)
  - *tetragonum* (Wither.) Ag. (30)
    - f. *tetragonum*
    - f. *brachiatum* (Bonnem.) K. Rosenv.
  - *tetricum* Ag.
- Ceramium Roth, 1797. (33)
  - *acanthonotum* Carm. ex Harv.
  - *arborescens* J. Ag.
  - *areschougii* Kylin
  - *atlanticum* Peterson
  - *boergesenii* Peterson
  - *ciliatum* (Ellis) Ducluz.
  - *circinatum* (Kütz.) J. Ag.
  - ? *crouanianum* J. Ag.
  - *derbesii* Solier
  - *deslongchampsii* Chauv.
  - *diaphanum* (Lightf.) Roth
  - *echionotum* J. Ag.
    - var. *echionotum*
    - var. *transcurrens* (Kütz.) Batt.
  - *fastigiatum* Harv.
  - *flabelligerum* J. Ag.
  - *fruticulosum* (Kütz.) J. Ag.
  - *gracillimum* Griff. et Harv.
  - *pedicellatum* (Duby) J. Ag.
  - *pennatum* Crn.
  - *rubrum* (Huds.) Ag.
  - *secundatum* J. Ag.
  - *strictum* Harv.
  - *tenue* J. Ag.
  - *tenuissimum* (Lyngb.) J. Ag.
  - *vimineum* J. Ag.
- Compsothamnion Nägeli, 1861.
  - *gracillimum* (Harv.) Näg.
  - *thuyoides* (Sm.) Näg.
- Corynospora J. Agardh emend. J. Agardh, 1876.
  - *pedicellata* (Sm.) J. Ag.
    - (*Monospora pedicellata* (Sm.) Solier)
    - f. *pedicellata*
    - f. *comosa* (Holm. et Batt.)
- Crouania J. Agardh, 1842.
  - *attenuata* (Bonnem.) J. Ag.
- Griffithsia Agardh, 1817.
  - *barbata* (Sm.) Ag.
  - *corallinoides* (L.) Batt.
  - *devoniensis* Harv. (34)
  - *flosculosa* (Ellis) Batt.
- Halurus Kützing, 1849.
  - *equisetifolius* (Ag.) Kütz.
    - var. *equisetifolius*
    - var. *simplicifilum* J. Ag.
- Microcladia Greville, 1830.
  - *glandulosa* (Soland.) Grev.
- Pleonosporium Nägeli emend. Nägeli ex Hauck, 1883.
  - *borreri* (Sm.) Näg. ex Hauck
    - var. *borreri*
    - var. *fasciculatum* (Harv.) Holm. et Batt.
- ? Plumaria Schmitz, 1896. (35)
  - ? *elegans* (Bonnem.) Schm.
- ? Ptilota Agardh, 1817. (35)
  - ? *plumosa* (Huds.) Ag.
- Ptilothamnion Thuret, 1863.
  - ? *lucifugum* Cotton (36)
  - *pluma* (Dillw.) Thur.
- Seirospora Harvey, 1846. (37)
  - *griffithsiana* Harv.
  - *hormocarpa* (Holm.) Batt.
  - *interrupta* (Sm.) Schm.
- Spermothamnion Areschoug, 1877. (38)
  - *barbatum* (Ag.) Näg.
  - *irregulare* (J. Ag.) Ardiss.
  - *repens* (Dillw.) K. Rosenv.
    - (*roseolum* Pringsh.)
    - (*turneri* (Mert.) Aresch.)
  - *strictum* (Ag.) Ardiss.
- Sphondylothamnion Nägeli, 1861.
  - *multifidum* (Huds.) Näg.
    - var. *multifidum*
    - var. *piliferum* (Ag.) Batt.
- Spyridia Harvey, 1833.
  - *filamentosa* (Wulf.) Harv.

Delesseriaceae
Acrosorium Zanardini, 1869.
reptans (Crn.) Kylin (39)
uncinatum (J. Ag.) Kylin (39)
Apoglossum J. Agardh, 1898.
ruscifolium (Turn.) J. Ag.
Cryptopleura Kützing, 1843.
ramosa (Huds.) Kylin
f. *ramosa*
f. *ciliifera* (Kütz.)
f. *lobata* (Kütz.)
f. *uncinata* (Grev.)
Delesseria Lamouroux, 1813.
sanguinea (Huds.) Lamour.
Erythroglossum J. Agardh, 1898.
sandrianum (Zanard.) Kylin
Gonimophyllum Batters, 1892.
buffhamii Batt.
Hypoglossum Kützing, 1843.
woodwardii Kütz. (40)
Membranoptera Stackhouse, 1809.
alata (Huds.) Stackh.
Nitophyllum Greville, 1830. (41)
? *bonnemaisonii* Grev.
(*Myriogramme bonnemaisonii* Kylin)
var. *bonnemaisonii*
var. *crassinerve* Batt.
punctatum (Stackh.) Grev.
? *versicolor* Harv.
(*Myriogramme versicolor* Kylin)
Pantoneura Kylin, 1919.
angustissima (Turn.) Kylin
Phycodrys Kützing, 1843.
rubens (Huds.) Batt.
Polyneura Kylin, 1924.
gmelinii (Grev.) Kylin
hilliae (Grev.) Kylin
litterata (J. Ag.) Kylin
Rhizoglossum Kylin, 1924.
thysanorhizans (Holm.) Kylin

Dasyaceae
Dasya Agardh, 1824.
arbuscula (Dillw.) Ag.
var. *arbuscula*
var. ? *caespitosa* J. Ag.
corymbifera J. Ag.
ocellata (Grat.) Harv.
punicea Menegh.
Heterosiphonia Montagne, 1842.
plumosa (Ellis) Batt.
f. *plumosa*
f. *patens* (Grev.)
f. *tenuior* (Dillw.)

Rhodomelaceae (42)
Bostrychia Montagne, 1838.
scorpioides (Huds.) Mont.
Brongniartella Bory, 1822.
byssoides (Good. et Woodw.) Schm.
Chondria Agardh, 1817.
caerulescens J. Ag.
dasyphylla (Woodw.) Ag.
tenuissima (Good. et Woodw.) Ag.
Halopitys Kützing, 1843.
incurvus (Huds.) Batt.
Laurencia Lamouroux, 1813. (43)
hybrida (D.C.) Lenorm. ex Duby
(*caespitosa* Lamour.)
obtusa (Huds.) Lamour.
pinnatifida (Huds.) Lamour.
Lophosiphonia Falkenberg, 1897.
obscura (Ag.) Fkbg.
(*Polysiphonia obscura* J. Ag.)
Odonthalia Lyngbye, 1819.
dentata (L.) Lyngb.
Polysiphonia Greville, 1824. (44)
brodiaei (Dillw.) Grev.
denudata (Dillw.) Kütz.
(*variegata* (Ag.) Zanard.)
elongata (Huds.) Harv.
elongella Harv.
fibrata (Dillw.) Harv.
fibrillosa (Dillw.) Grev. (45)
foetidissima Cocks
fruticulosa (Wulf.) Spreng.
(*Pterosiphonia fruticulosa* (Wulf.) Batten)
furcellata (Ag.) Harv.
var. *furcellata*
var. *forcipata* J. Ag.
insidiosa Crn.
lanosa (L.) Tandy (*fastigiata* Grev.)
macrocarpa Harv.
nigra (Huds.) Batt.
nigrescens (Sm.) Grev.
f. *nigrescens*
f. *affinis* (Moore) J. Ag.
f. *fucoides* (Huds.) J. Ag.
f. *protensa* J. Ag.
f. *senticosa* J. Ag.
opaca (Ag.) Zanard.
rhunensis Thur.
richardsonii Hook.
? *sanguinea* (Ag.) Zanard.
simulans Harv.
spinulosa Grev.
var. *spinulosa*
var. *major* J. Ag.
spiralis Batten
subulifera (Ag.) Harv.
var. *subulifera*
var. *templetonii* Harv.
urceolata (Dillw.) Grev.
f. *urceolata*
f. *comosa* (Ag.) J. Ag.
f. *formosa* (Suhr) J. Ag.
f. *patens* (Dillw.) J. Ag.
violacea (Roth) Grev. (46)
Pterosiphonia Falkenberg, 1889.
complanata (Clem.) Fkbg.
parasitica (Huds.) Fkbg.
pennata (Roth) Fkbg.
thuyoides (Harv.) Schm.
Rhodomela Agardh, 1822. (47)
confervoides (Huds.) Silva
(*subfusca* (Woodw.) Ag.)
lycopodioides (L.) Ag.

Notes on Rhodophyceae

(1) See Waern (1952).

(2) See Hamel (1924, p. 283).

(3) British material of genus in need of revision.

(4) According to Koster (1952) should be *B. atropurpurea* (Roth) Ag. f. *fuscopurpurea* (Dillw.) Ag. as there are no differences to be found between the fresh-water and marine material; see also Hamel (1924), and Kylin (1944). Kept the marine form distinct for the present awaiting the results of Dr K. M. Baker's investigations.

(5) See Drew (1949).

(6) *Acrochaetium* Nägeli, sens. lat.

(7) Dr K. M. Baker is revising the British material of this genus.

(8) Awaiting the results of the investigation of this genus carried out by Dr M. de Valèra and Mr P. Dixon.

(9) Requires investigation.

(10) *Nemastoma* J. Agardh, 1842; *N. dichotoma* J. Ag., recorded by Lyle (1920) for Guernsey. J. Feldmann (1941) thinks that several species may have been confused under the above name, so Lyle's specimen should be examined.

(11) See Papenfuss (1950), Waern (1952), Silva (1952).

(12) *Rhodophyllis bifida* var. *incrassata* Harv., requires investigation before any new combination is made under *R. divaricata. R. appendiculata* may be a form of *R. divaricata* (J. Feldmann, 1941).

(13) See J. Feldmann (1941, p. 86) and G. Schotter (1952).

(14) The form-range of the species on the British coast require investigation.

(15) See Marshall, Newton & Orr (1949).

(16) British material belonging to this family requires monographic treatment.

(17) Systematic position uncertain.

(18) See Cotton (1912).

(19) See Kylin (1944).

(20) In need of monographic treatment.

(21) Needs investigation—should be possibly *C. mediterranea* Aresch. or *C. officinalis* L. var. *mediterranea* (Aresch.) Hauck.

(22) The genus *Fosliella* Howe not adopted for the present—appears to require further investigation. If adopted the British species would be *F. farinosa* (Lamour.) Howe, *F. lejolisii* (Rosan.) Howe, *F. minuta* (Fosl.), *F. zonalis* (Crn.) J. Feldm., and *Epilithon membranaceum* would again be known as *Melobesia membranacea* (Esper.) Lamour.

(23) Should possibly be placed in genus *Pseudolithothamnion* Lemoine, 1913; see Lemoine (1928).

(24) Regarded by some workers as a form of *L. glaciale.*

(25) *K. larterae* Holm. requires investigation—may be a form of *K. reniformis* (Turn.) J. Ag.

(26) See J. & G. Feldmann (1942, 1952) and J. Feldmann (1952).

(27) The form-range of the species requires investigation and revision.

(28) Possibly a form of *A. spirographidis,* see J. Feldmann (1942).

(29) British species require investigation as no doubt some of the species listed here under *Callithamnion* should be placed under *Aglaothamnion.* The range of form of species of this genus on the British coast is in urgent need of attention.

(30) Kolderup Rosenvinge (1924) and Kylin (1944) consider *C. tetragonum, C. spiniferum* Kylin (Dutch coast) and *C. fruticulosum* forms of one species. Kolderup Rosenvinge (1924) unites them under *C. tetragonum* but Kylin (1944) prefers to keep them separate and to use the name *C. tetragonum* for plants on the British coast and *C. fruticulosum* for the plants on the Swedish coast. The form-range of the series on the British coast needs investigation and comparison with material from Sweden, Holland and Denmark.

(31) Cotton (1912) thinks that *C. spongiosum* and *C. granulatum* may be two distinct species, having found no intermediate forms between them. They have been listed separately awaiting monographic treatment of British material.

(32) Left as *C. roseum* Harv. until it is certain that Harvey's plant is the same as *C. roseum* (Roth) Lyngb.

(33) British material in urgent need of revision, see work of Petersén, Kylin, Sjösted), Waern (references to literature in Waern, 1952), Kolderup Rosenvinge (1924), G. Feldmann-Mazoyer (1940), Lucas (1950). The different workers' interpretations of many of the species and of the form-range within any one species vary so much that no attempt has been made to link up the 'species' recorded for the British coast with these treatments. This must await monographic treatment of British material.

(34) Possibly an ecological form of *G. corallinoides*, Koster (1952).

(35) *Plumaria* Schm. and *Ptilota* Ag. used awaiting decision concerning conservation of *Plumaria* Schm. over *Plumaria* Stackh.—see Silva (1952).

(36) See Cotton (1912), G. Feldmann-Mazoyer (1940); *P. lucifugum* Cotton may be a form of *P. pluma* since G. Feldmann considers that *P. micropterum* (Mont.) Born. is a reduced form of *P. pluma*. Fertile material of Cotton's plant needs investigation.

(37) British material in need of revision.

(38) British material requires investigation and revision. Kolderup Rosenvinge places *S. turneri* (Mert.) Aresch. as var. *turneri* (Mert.) K. Rosenv. and *S. roseolum* (Ag.) Pringsh. as var. *roseolum* (Ag.) K. Rosenv. under *S. repens* (Dillw.) K. Rosenv. If *Conferva repens* Dillw. is the same plant as that described by Mertens as *Ceramium turneri*, which Kolderup Rosenvinge believed it was, then var. *turneri* should be placed as a synonym under *S. repens* (Dillw.) K. Rosenv. var. *repens*. See J. Feldmann (1942, p. 59) concerning *S. strictum*.

(39) Doubtful if these two species are distinct.

(40) Forms of this species occurring on British coast in need of revision.

(41) Kylin (1934) considered that *N. bonnemaisonii* and *N. versicolor* are closer to the genus *Nitophyllum* than to the genus *Myriogramme* Kylin and so should remain in the genus *Nitophyllum* for the present; he considered, however, that they are sufficiently different from the type species *N. punctatum* to warrant their being placed in a new genus.

(42) *Ctenosiphonia* Falkenberg, 1897; *C. hypnoides* (Welw.) Fkbg. recorded for Guernsey by Lyle (1920, 1923).

(43) Form-range of species on British coast needs revision.

(44) Form-range of species on British coast requires investigation.

(45) Considered by some workers a form of *P. violacea*; British material needs investigation.

(46) The forms of this species on the British coast need investigation, see Kolderup Rosenvinge (1924), Kylin (1944), Veldkamp (1950). *P. subulata* (Ducluz.) J. Ag. considered a distinct species by some workers and not a variety of *P. violacea*.

(47) Forms of *R. confervoides* require revision before any new combinations are made. *R. lycopodioides* should possibly be included as a variety under *R. confervoides*.

CYANOPHYCEAE (1)

Chroococcaceae (2)
ANACYSTIS Meneghini, 1837.
  marina (Hansg.) Dr. et Daily
    (*Aphanocapsa marina* Hansg.)
  ? *parasitica* Kütz.
? APHANOCAPSA Nägeli, 1849.
  ? *litoralis* Hansg.
? APHANOTHECE Nägeli, 1849.
  ? *pallida* (Kütz.) Rabenh.
? CHROOCOCCUS Nägeli, 1849.
  ? *calcicola* Anand
  ? *turgidus* (Kütz.) Näg.
? GLOEOCAPSA Kützing, 1843.
  ? *crepidinum* Thur.
? GLOEOTHECE Nägeli, 1849.
  ? *palea* (Kütz.) Rabenh.
? HOLOPEDIA Lagerheim, 1883.
  ? *sabulicola* (Lagerh.) Kirchn.
MERISMOPEDIA Meyen, 1839.
  ? *convoluta* Bréb. ex Kütz.
  ? *glauca* (Ehrb.) Näg.
    f. *glauca*
    f. ? *mediterranea* (Näg.) Collins
  ? *revoluta* Ask.
? SYNECHOCOCCUS Nägeli, 1849.
  ? *bacillaris* Butcher

Chamaesiphonaceae (2, 3)
? CHAMAESIPHON A. Braun. et Grunow, 1865.
  ? *marinus* Wille (4)
? CHLOROGLOEA Wille, 1900.
  ? *tuberculosa* (Hansg.) Wille
? DERMOCARPA Crouan, 1858.
  ? *enteromorphae* Anand
  ? *leibleiniae* (Reinsch) Born. et Thur.
  ? *olivacea* (Reinsch) Tilden
  ? *incrustans* (Reinsch) Batt.
  ? *rosea* (Reinsch) Batt.
  ? *sphaerica* Setch. et Gardn. ex Gardn.

- ? DERMOCARPA (*cont.*)
  - ? *violacea* Crn.
- ENTOPHYSALIS Kützing, 1843.
  - *conferta* (Kütz.) Dr. et Daily
    - (*Dermocarpa prasina* (Reinsch) Born. et Thur.)
    - (*Xenococcus schousboei* Thur.)
  - *crustacea* (Ag.) Dr. et Daily
    - (*granulosa* Kütz.)
    - (*Hyella caespitosa* Born. et Flah.)
    - (*Pleurocapsa fuliginosa* Hauck)
- ? ONCOBYRSA Agardh, 1827.
  - ? *marina* (Grun.) Rabenh.
- ? PLEUROCAPSA Thuret, 1885.
  - ? *amethystea* K. Rosenv.
  - ? *crepidinum* Collins
  - ? *entophysaloides* Setch. et Gardn. ex Gardn.
  - ? *kerneri* (Hansg.) Dr.
- ? XENOCOCCUS Thuret ex Bornet et Thuret, 1880.
  - ? *gilkeyae* Setch. et Gardn. ex Gardn. (forma)
  - ? *pyriformis* Setch. et Gardn. ex Gardn.
  - ? *violacea* Anand

Stigonemataceae

- BRACHYTRICHIA Zanardini; Bornet et Flahault, 1886.
  - *balani* (Lloyd) B. et F.
- MASTIGOCOLEUS Lagerheim; Bornet et Flahault, 1887.
  - *testarum* Lagerh. ex. B. et F.

Nostocaceae

- ANABAENA Bory; Bornet et Flahault, 1888.
  - ? *berkeleyana* (Thwaites) Batt.
  - ? *broomei* (Thwaites) Batt.
  - *torulosa* (Carm.) Lagerh. ex B. et F.
  - *variabilis* Kütz. ex B. et F.
- NODULARIA Mertens; Bornet et Flahault, 1888.
  - *harveyana* (Thwaites) Thur. ex. B. et F.
  - *spumigena* Mert. ex. B. et F.
    - var. *litorea* (Kütz.) B. et F.
- NOSTOC Vaucher; Bornet et Flahault, 1888.
  - *commune* Vauch. ex B. et F.
  - *entophytum* B. et F.
  - *linckia* (Roth) Born. ex B. et F.
- PSEUDANABAENA Lauterborn, 1916.
  - *brevis* Carter

Rivulariaceae

- AMPHITHRIX Kützing; Bornet et Flahault, 1886.
  - *violacea* (Kütz.) B. et F.
- CALOTHRIX Agardh; Bornet et Flahault, 1886.
  - *aeruginea* (Kütz.) Thur. ex B. et F.
  - *confervicola* Ag. ex B. et F.
    - var. *confervicola*
    - var. *purpurea* B. et F.
  - ? *consociata* (Kütz.) B. et F. (5)
  - ? *contarenii* B. et F. (6)
  - *cottonii* nov. nom. (7)
    - (*endophytica* Cotton)
  - *crustacea* Thur. ex B. et F.
  - *fusca* (Kütz.) B. et F.
  - *litoralis* Anand
  - *parasitica* (Chauv.) Thur. ex. B. et F.
  - *pulvinata* (Ag.) B. et F.
    - var. *pulvinata*
    - var. *prostrata* Anand
  - *scopulorum* (Web. et Mohr) Ag. ex B. et F.
    - (*fasciculata* Ag. ex B. et F.) (8)
  - *vivipara* Harv. ex B. et F.
- DICHOTHRIX Zanardini; Bornet et Flahault, 1886.
  - *gypsophila* (Kütz.) B. et F.
- ISACTIS Thuret; Bornet et Flahault, 1886.
  - *plana* Thur. ex B. et F.
    - var. *plana*
    - var. *fissurata* B. et F.
- RIVULARIA Roth; Bornet et Flahault, 1886.
  - *atra* Roth ex B. et F. (9)
  - *australis* Harv. ex B. et F.
  - *biasolettiana* Menegh. ex B. et F.
  - *bullata* (Poir.) Berk. ex B. et F.
  - *mesenterica* Thur. ex B. et F.
  - *nitida* Ag. ex B. et F. (9)

Scytonemataceae

- ? DIPLOCOLON Nägeli; Bornet et Flahault, 1887. (10)
  - ? *codii* Batt. (10)
- FREMYELLA J. De Toni, 1936.
  - (MICROCHAETE Thuret, 1875)
  - *aeruginea* (Batt.) nov.comb.
    - (*Microchaete aeruginea* Batt.)
  - *grisea* (Thur. ex B. et F.) J. De Toni
    - (*Microchaete grisea* Thur.)
- PLECTONEMA Thuret; Gomont, 1893.
  - *battersii* Gom.
  - *litorale* Anand
  - *norvegicum* Gom.
  - *nostocorum* Born. ex Gom.
  - *terebrans* Born. et Flah. ex Gom.
- TOLYPOTHRIX Kützing; Bornet et Flahault, 1887.
  - *tenuis* Kütz. ex B. et F.

Oscillatoriaceae

- HYDROCOLEUM Kützing; Gomont, 1892.
  - *glutinosum* (Ag.) Gom.
  - *lyngbyaceum* Kütz. ex Gom.
    - var. *lyngbyaceum*
    - var. *rupestre* Kütz. ex Gom.
- LYNGBYA Agardh; Gomont, 1893.
  - *aestuarii* Liebm. ex Gom. (11)
    - f. *aestuarii*
    - f. *aeruginosa* Gom.
    - f. *ferruginea* Gom.
    - f. *limicola* Gom.
    - f. *natans* Gom.

LYNGBYA (*cont.*)
aestuarii (*cont.*)
f. *spectabilis* Gom.
f. *symplocoidea* Gom.
agardhii Gom.
confervoides Ag. ex. Gom.
gracilis Rabenh. ex Gom.
var. *maritima* Anand
lutea Gom. ex Gom.
majuscula Harv. ex Gom.
meneghiniana Gom. ex Gom.
rivulariarum Gom.
semiplena J. Ag. ex Gom.
MICROCOLEUS Desmazières; Gomont, 1892.
acutirostris Gom.
chthonoplastes Thur. ex Gom.
tenerrimus Gom.
OSCILLATORIA Vaucher; Gomont, 1893. (12)
amphibia Ag. ex Gom.
bonnemaisonii Crn. ex Gom.
brevis Kütz. ex Gom.
var. *neapolitana* Gom.
corallinae Gom. ex Gom.
formosa Bory ex Gom.
laetevirens Crn. ex Gom.
limosa Ag. ex Gom.
margaritifera Kütz. ex Gom.
nigro-viridis Thwaites ex Gom.
sancta Kütz. ex Gom.
PHORMIDIUM Kützing; Gomont, 1893.
ambiguum Gom.
angustissimum W. et G. S. West
autumnale Gom.
corium Gom.
ectocarpi Gom.
foveolarum Gom.
f. *foveolarum*
f. *minus* Anand
f. *moniliforme* Anand
fragile Gom.
monile Setch. et Gardn. (forma)
papyraceum Gom. ex Gom.
persicinum Gom.
submembranaceum Gom.
var. *minor* Anand
subuliforme Gom.
tenue Gom.
uncinatum Gom. ex Gom.
valderianum Gom.
SCHIZOTHRIX Kützing; Gomont, 1892.
cresswellii Harv. ex Gom.
fritschii Anand
lardacea Gom.
vaginata Gom.
SPIRULINA Turpin; Gomont, 1893.
major Kütz. ex Gom.
subsalsa Oerst. ex Gom.
f. *subsalsa*
f. *oceanica* Gom.
subtilissima Kütz. ex Gom.
versicolor Cohn ex Gom. (13)
SYMPLOCA Kützing; Gomont, 1893.
atlantica Gom.
var. *atlantica*
var. *purpurea* Batt.
dubia Gom.
hydnoides Kütz. ex Gom.
var. *hydnoides*
var. *fasciculata* Gom.

Notes on Cyanophyceae

(1) For various systems of classification see Geitler (1932), Frémy (1934), Fritsch (1945), Smith (1950); families only given in list.

(2) See Drouet (1951) and Drouet & Daily (1952) who are also preparing for publication a detailed revision of the coccoid Cyanophyceae. Dr F. Drouet has very kindly given the following information for inclusion here. In the revision of the Chroococcaceae the genus *Anacystis* Menegh. will include *Aphanocapsa*, *Chroococcus* and *Gloeocapsa* of this list and the genus *Coccochloris* Spreng. will include *Aphanothece*, *Gloeothece*, and *Synechococcus* of this list. In the revison of the Chamaesiphonaceae the genus *Entophysalis* Kütz. will include all the genera given under this family in this list including *Pleurocapsa* Thur. as to type, but not as to many of the species which largely are forms of *Anacystis*.

(3) See also Nadson (1932).

(4) Probably not a member of the Cyanophyceae.

(5) =*C. confervoides* Ag.?

(6) Possibly a form of *C. scopulorum* Ag.

(7) =*Calothrix endophytica* Cotton, *Proc. Roy. Irish Acad.*, Vol. 31, p. 104, 1912 [non *Calothrix endophytica* Lemmerman, *Forsch. Ber. d. Biolog. Stat. zu Plön*, p. 184, 1896=*Homoeothrix endophytica* (Lemm.) Lemm., *Krypt. Flor. Mark Brandenburg*, Bd. 3, p. 240, 1910.]

(8) Both Lindstedt (1943) and Du Rietz (1947) consider that *C. fasciculata* Ag. is a luxuriant growth form of *C. scopulorum*. The plant figured and described as *C. fasciculata* by Harvey in Phyc. Brit., may be, according to some authorities, a species quite distinct from the true *C. fasciculata* Ag.

(9) Geitler (1932) suggests that *R. atra* is possibly the young stage of *R. nitida*.

(10) The value of this genus appears to be debatable since *D. heppii* Näg., on which the genus was founded, is, according to some authorities, only a form of *Scytonema crustaceum* Ag. There also seems to be doubt concerning Batters' plant (Frémy, 1934) since his diagrams do not show false branching but true branching—this requires investigation.

(11) Intermediates occur between all the described forms (Frémy, 1934).

(12) Two species omitted from list as they are in need of investigation. (*a*) *O. rosea* (Crn.) Batt. [non *O. rosea* Utermöhl, 1925, = *O. utermoehlii* J. De Toni, 1939] given as a sp. inquir. in Gomont. (*b*) *O. subuliformis* Thwaites ex Harv., Phyc. Brit.; does Thwaites' plant = *O. subuliformis* Kütz.?

(13) Probably a form of *S. subsalsa*—no essential difference between the two species (Geitler, 1932).

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

RECEPTOR ELEMENTS IN THE THORACIC MUSCLES OF *HOMARUS VULGARIS* AND *PALINURUS VULGARIS*

By J. S. Alexandrowicz

Quart. Journ. Micr. Sci., Vol. 93, 1952, pp. 315–46

In the thoracic muscles of *Homarus vulgaris* and *Palinurus vulgaris* the presence of receptor elements of various kinds has been recorded. Muscle receptor organs belonging to the same category as described previously in the abdomen have been found in the two posterior (7th and 8th) thoracic segments. Like those in the six abdominal segments they are linked with the system of the extensor muscles. The topography and the structure of these organs have been described.

Nerve cells regarded as receptors of a different category have been found in some of the muscles inserting in the median surface of the epimeral plate. These cells, termed '*N*-cells', are smaller elements than those of the first category and have not a special muscle of their own, but end with long processes between the fibres of the ordinary muscles. In each of the species investigated five such elements have been found. It is suggested that the *N*-cells may represent more primitive forms of muscle receptors, and the receptor organs of the extensor muscles in the thorax and abdomen are more highly evolved forms. J.S.A.

NOTES ON THE NERVOUS SYSTEM IN THE STOMATOPODA.

II. THE SYSTEM OF DORSAL TRUNKS.
III. SMALL NERVE CELLS IN MOTOR NERVES

By J. S. Alexandrowicz

Pubbl. Staz. Zool. Napoli, Vol. 24, 1953, pp. 29–45

On the ventral surface of the extensor muscles in *Squilla mantis* a pair of nerve trunks has been found which run longitudinally through the thoracic and abdominal segments. In each of the first five abdominal segments there are four ganglion cells in each trunk. In the same segments five paired nerves arise from the trunks and give off branches ending in two neuropile-like networks situated in the pericardial cavity: the first in its lateral wall and the second in a lamella spanned between the dorsal muscles of the opposite sides. It is suggested that these nervous elements have a neurosecretory function.

In the motor nerves running on the ventral surface of the extensor muscles in *Squilla mantis* nerve cells of small size have been found. They have long processes running alongside the motor fibres. There is no evidence of any relations of these cells with the system of dorsal trunks. J.S.A.

RESISTENZ DER MEERESALGEN GEGEN SICHTBARES LICHT UND GEGEN KURZWELLIGE UV-STRAHLEN

By **R. Biebl**

Protoplasma, Bd. 41, 1952, pp. 353–77

The tissues of marine algae show a typical 'ecological resistance' to direct sunlight. After 2 hr. exposure to sunlight or 3–5 days' exposure to two 350 W. electric-light bulbs all the cells of sublittoral algae were killed, while those of intertidal algae remained intact.

The degree of resistance to ultra-violet light is characteristic for algae within each ecological zone (intertidal, low-water, or sublittoral): it is constitutional, and not conditioned by the environment. Resistances to sunlight and to ultra-violet light are different characteristics of the tissues.

Photographs of dead cells show that those killed by visible light are in general rapidly bleached, in contrast to those killed by ultra-violet light or other deleterious agents. R.B.

LICHTTRANSMISSIONSÄNDERUNGEN AN MEERESALGEN, IM BESONDEREN AN *PORPHYRA UMBILICALIS* F. *LACINIATA*

By **R. Biebl**

Oesterr. botan. Zeitschr., Bd. 100, 1953, pp. 179–202

The light-transmission between 400 and 800 mμ was measured with an Unicam Photoelectric Quartz Spectrophotometer on small pieces of the red alga *Porphyra umbilicalis* (L.) Kütz. f. *laciniata* (Lightf.) J.Ag. in order to compare the values obtained before and after various treatments.

If *Porphyra*, after being kept for 17 hr. in hypotonic (10% normal) or hypertonic (300% normal) sea water, is desiccated for 3 hr. or exposed for 2 hr. to direct sunlight, it exhibits a remarkable raising of light-transmission, which is fully reversible if the alga is brought back to normal conditions for about 20 hr. If the pieces are put in darkness after 2 hr. exposure to sunlight, the light transmission continues to rise for about 5 hr., but afterwards returns gradually to the original values. Heat (34° C.) also causes raising of light-transmission. Cold (−2° C.) does not.

There is a certain contrast between the extinction curves obtained with algae treated with distilled water or 10% sea water on the one hand, and those treated with concentrated sea water, or desiccated, on the other. For descriptive purposes, the terms 'swelling type' (Quellungstyp) and 'shrinking type' (Entquellungstyp) have been adopted.

It was not possible to study the cause of changes in light transmission in the time available. Alterations in the form of the big plastids as well as changes in the colloidal structure of a reversible nature in the plastids ought to be taken into consideration. It is unlikely that the pigments could be decomposed and resynthesised during the short duration of the experiments.

R.B.

SOME STRUCTURAL PROTEINS OF *MYTILUS EDULIS*

By C. H. Brown

Quart. Journ. Micr. Sci., Vol. 93, 1952, pp. 487–502

The byssus threads, periostracum, hinge and ground substance of the shell and the supporting material of the gills of *Mytilus edulis* were examined by physical, chemical and histochemical means. The byssus threads, periostracum and hinge were shown to consist of a quinone-tanned protein. The byssus threads are formed in the posterior groove of the foot from the secretions of two glands, the 'white' gland which supplies the bulk of the protein of the thread and the 'purple' gland which supplies the aromatic material responsible for the tanning. The periostracum is secreted by gland cells in the epithelium of the outer lobe of the mantle edge. The supporting material of the gills is a fibrous protein without quinone-tanning. This protein is in some respects similar to the untanned protein of the byssus. C.H.B.

PRESENZA DI SPICOLE IN *DIPLOSOMA LISTERIANUM* (MILNE EDWARDS). CONTRIBUTO ALLA SISTEMATICA DEGLI ASCIDIACEA, DIDEMNIDAE (THE PRESENCE OF SPICULES IN *DIPLOSOMA LISTERIANUM* [MILNE EDWARDS]. A CONTRIBUTION TO THE SYSTEMATICS OF THE ASCIDIACEA, DIDEMNIDAE)

By D. B. Carlisle

Pubbl. Staz. Zool. Napoli, Vol. 24, 1953, pp. 62–8

The presence of calcareous spicules is reported in the test of some colonies of this species collected at Naples and in a much less proportion of colonies collected at Plymouth. They are much smaller than is usual in the Didemnidae and are rapidly destroyed by the trace of formic acid present in formalin so that they are normally absent from preserved material. They are invisible except with phase contrast when a piece of test is mounted in canada balsam. The nomenclature of the species is discussed and it is suggested that the report of these spicules destroys the last remaining difference between the genera *Lissoclinum* and *Diplosoma*, so that they should now be merged under the older name—*Diplosoma* Macdonald. It is possible that *Lissoclinum pseudoleptoclinum* (Drasche) may be a subspecies of *Diplosoma listerianum*.

D.B.C.

Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). II. Experimental Evidence for a Growth- and Moult-Accelerating Factor obtainable from Eyestalks

By **D. B. Carlisle** and **P. F. R. Dohrn**

Pubbl. Staz. Zool. Napoli, Vol. 24, 1953, pp. 69–83

A solution made by extracting the eyestalks of fast-moulting (summer) female *Lysmata* or *Palaemon* spp. with distilled water acidified to pH 3·5–3·8 with hydrochloric acid was active in accelerating the rate of moulting when injected intramuscularly into *Lysmata*. There was no significant activity in male eyestalk extracts or in extracts made from eyestalks taken from females in winter when the moult rate is very low. The active material was destroyed by boiling. The moult rate was also accelerated by vertebrate pituitary extracts and crude human chorianic gonadotrophin. It is suggested that the active material in the eyestalk extracts is a hormone whose site of origin is probably neurosecretory cells in the central nervous system of the eyestalk and other ganglia. It is concluded that the initiation of the moulting process may be under the control of the moult-inhibiting hormone, but the process of the premoult, once begun, is controlled by the moult-accelerating hormone.

D.B.C.

Plankton of the Benguela Current

By **T. J. Hart**

Nature, Vol. 171, 1953, pp. 631–4

Preliminary results from net-caught phytoplankton samples showed good agreement with the hydrological results described in a previous article by Currie, and some analogy with conditions off California. Many cosmopolitan species seemed especially abundant in both areas. In the Benguela, chaetocerids predominated in the richest coastal area both in autumn and in spring. *Planktoniella* formed a good indicator of the much poorer offshore current at both seasons, and followed intrusions of this water into the area of the upwelling current very closely, as Gunther had found off Peru. *Goniaulax spinifera* was dominant in a localized area where lateral mixing between the two main types of surface waters seemed most probable, but the total plankton there was not rich. Many individual species of diatoms showed distributional patterns conforming to the disposition of the water masses. The population of the rich inshore area tended to be greater in autumn than in spring, and this is thought to be due to the lessened upwelling intensity in autumn, some time-lag favouring maximum development of the succession in the previously upwelled water.

Samples of discoloured surface-water near Walvis Bay at the time of a moderate fish mortality showed *Peridinium triquetrum* dominant in reddish areas (3–6 million cells/l.). Khaki areas were less rich (some half-million cells/l.) and contained many diatoms (*Asterionella*) and *Prorocentrum* in addition to the *P. triquetrum*. Water bloom of several different types is now known to occur in the region, though blooms due to *Gymnodinium* or *Goniaulax*, such as are known to be lethal elsewhere, have not yet been identified. It is suggested hypothetically that the fish mortalities may result from multiple causes, with the emphasis on lack of oxygen in the subsurface layers, rather than from the action of noxious dinoflagellates alone.

Some outstanding features of the zooplankton distribution are also briefly mentioned. T.J.H.

CONTRACTION AND RELAXATION IN THE ADDUCTOR MUSCLES OF *MYTILUS EDULIS*

By J. Lowy

Journ. Physiol., Vol. 120, 1953, pp. 129–40

The spontaneous electrical and mechanical activity in the intact posterior adductor muscle of *Mytilus edulis* has been recorded simultaneously and continuously for long periods of time with the animal in water or exposed to air. It was found that both phasic and tonic contractions of the adductor are accompanied by muscle action potentials. Tonic contraction is associated with intermittent excitatory volleys as well as with electrical activity in the muscle during the intervals between volleys.

Experiments with preparations of the posterior adductor muscle show that nerve elements in the visceral ganglia control the duration and termination of a state of contraction. On the present evidence no explanation can as yet be given about the mechanism involved in the nervous control of relaxation.

When placed in oxygenated sea water the denervated posterior adductor muscle relaxes within a few hours. Strips of this muscle can also be isolated in a relaxed state. Such preparations remain excitable to electrical stimuli and exert a maximum tension for up to two days at 14° C. J.L.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1952–53

Patron

The Council has pleasure in reporting that His Royal Highness The Duke of Edinburgh, K.G., has graciously consented to become Patron of the Marine Biological Association of the United Kingdom.

The Council and Officers

Four meetings of the Council have been held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was seventeen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

The Council has to record with regret the deaths of two Vice-Presidents, Viscount Astor and Dr W. T. Calman, C.B., F.R.S.; of Dr Th. Mortensen, an Honorary Member of the Association; and of Prof. J. H. Orton, F.R.S., sometime member of Council and for many years a member of the Staff of the Plymouth laboratory.

The Plymouth Laboratory

Following the completion last year of the building of the laboratory for research with radioactive substances, the opportunity has been taken of paving the area between this laboratory and the main building with concrete.

The facilities of the main workshop have been improved by the purchase of a milling machine.

The Council is grateful to the Development Commissioners for the supply of a new Morris 10 cwt. truck.

The Aquarium

Throughout the summer months the aquarium was crowded with visitors, and their appreciation and interest is reflected in the sale of large numbers of the aquarium guide book. Tanks have been stocked to capacity, perhaps the most notable exhibits being the wreck-fish (*Polyprion americanus*), mentioned in last year's report and now in fine condition, and about twenty boar-fish (*Capros aper*), which make an especially colourful display. Some of the sea-water mains which had corroded have been renewed and three new panes of glass have been inserted on the south side. Dr D. P. Wilson has published in Volume XXXI, No. 1, of the *Journal* an account of the aquarium and circulation system in use at Plymouth, with suggestions for improvement and some suggestions of application to all large marine aquaria.

Research Ships

During the year the piston and liner of one of the cylinders of the *Sabella* have been renewed, and it has been necessary to fit a new tail shaft and rudder spindle to the *Sula*. Both ships, and the *Gammarus*, are in good condition and have been working regularly throughout the year.

New Research Ship

The keel of the new research ship now being built in the yard of Messrs Philip and Son Ltd., at Dartmouth, was laid in August 1952, and work is proceeding according to schedule. Dr G. A. Steven and Captain C. A. Hoodless have given a considerable amount of time to consideration of the many details involved, and Mr F. J. Warren has given much assistance in the planning of the electrical installations.

The Staff

Dr J. A. C. Nicol has been promoted to the grade of Principal Scientific Officer.

By arrangement with University College, London, Mr B. C. Abbott joined the staff of the Plymouth Laboratory on 1 September 1952 on a special temporary appointment.

Dr H. W. Harvey, F.R.S., has been awarded the Agassiz Medal for 1952 by the National Academy of Sciences, Washington.

Mr F. S. Russell attended the forty-first meeting of the International Council for the Exploration of the Sea in Copenhagen in October 1952; and he and Dr Mary Parke attended the International Seaweed Symposium held in Edinburgh in July 1952.

Mr G. M. Spooner was awarded a grant by the Institut Océanographique to spend the month of June 1952 in the Musée Océanographique at Monaco.

Mr D. B. Carlisle was awarded a grant to spend the month of May 1952 at the Stazione Zoologica, Naples. At the end of August he went to the Stazione to assist in the Physiological Department for four months.

Occupation of Tables

The following one hundred and eight workers have occupied tables at the Plymouth laboratory during the year:

B. C. ABBOTT, London (Physiology of muscle).
E. ADAMS, Plymouth (Library).
J. A. ALLEN, Glasgow (Ciliary mechanism of Lucinacea).
Dr DAPHNE ATKINS, London (Biology of *Pinnotheres*).
K. H. BAIN, Gold Coast (Fisheries).
R. BAINBRIDGE, Oxford (Interrelationships of plankton organisms).
R. H. BAIRD, Conway (Oyster culture).
Miss D. BALLANTINE, Development Commission (Taxonomy and culture of flagellates).

I. D. Beall, Plymouth (Library).
Dr Anna M. Bidder, Cambridge (Cephalopods).
Dr H. Blaschko, Oxford (Enzymes in *Loligo*).
B. Boczarow, Plymouth (Library).
A. D. Boney, Plymouth (Algae).
E. R. Braithwaite, Plymouth (Library).
Dr Eleanor M. Brown, London (Parasites of copepods).
Prof. & Mme L. Brull, Liège (Physiology of kidney in *Lophius*).
G. O. Burgess, Hull (Epidermal secretions of fish).
Dr J. N. Carruthers, London (Bottom sampling).
P. Childs, Plymouth (Comparative anatomy of biliary tract).
Dr P. N. J. Chipperfield, Brixham (Library).
R. B. Clark, Glasgow (Behaviour of polychaetes).
Dr H. A. Cole, Conway (Oyster culture).
Miss M. Collis, London (Anatomy and physiology of *Sabellaria*).
C. A. Cosway, Torquay (Library).
C. B. Cowey, Oxford (Biochemistry).
R. I. Currie, National Institute of Oceanography (Oceanography).
L. Cuypers, Liège (Physiology of kidney in *Lophius*).
Dr R. Phillips Dales, London (Behaviour of *Amphitrite*).
Dr A. K. Das, India (Biochemistry of fish).
Dr D. Davenport, Santa Barbara, Cal., U.S.A. (Physiology of commensalism).
Prof. J. H. Day, Cape Town (General).
K. Eriksen, Oslo (General).
Dr Maria Felińska, Cheltenham (Ciliates).
L. R. Fisher, Reading (Vitamin A in cephalopods).
Miss V. E. Ford, Brighton (Algae).
Cdr R. H. C. F. Frampton, R.N. (Rtd.), Plymouth (Library).
Dr Vera Fretter, London (Osmoregulation in *Nereis diversicolor*).
B. L. Ginsborg, London (General).
Dr C. P. Gnanamuthu, Madras (General).
Dr Helen Goodrich, Oxford (*Phascolosoma*).
J. N. R. Grainger, Hull (Respiration in crustaceans).
Miss S. G. Greening, Birmingham (*Arenicola*).
Dr Antoinette Guelin, Paris (Bacteriophages in fishes).
D. N. F. Hall, Colonial Fisheries (Biology of decapods).
Miss M. B. Harley, London (Feeding habits of *Nereis*).
Dr T. J. Hart, National Institute of Oceanography (Plankton).
F. A. B. Hawes, London (Fouling organisms; settlement in *Tubularia*).
R. H. Hedley, Newcastle (Tube formation in serpulids).
Dr C. F. Hickling, Colonial Office (General).
Miss J. M. Himms, Oxford (Enzymes in *Loligo*).
Prof. A. L. Hodgkin, F.R.S., Cambridge (Giant nerve fibres in *Loligo*).
E. P. Hodgkin, Western Australia (General).
Dr L. B. Höglund, Uppsala (*Spirorbis*).
G. Hoyle, Glasgow (Spontaneous activity of tunicates).
O. D. Hunt, Newton Ferrers (Library).
R. F. Hutton, Miami, Fla., U.S.A (Fulbright Scholar) (Trematode parasites).
L. A. J. Jackman, Paignton (Library).
Miss M. W. Jarvis, London (*Hemimysis* and *Macromysis*).
F. J. Jeffery, Plymouth (Library).

L. W. G. JONES, Plymouth (Library).
Dr W. C. JONES, Cambridge (*Leucosolenia*).
K. A. & Mrs JOYSEY, London (Biometrics of *Echinus esculentus*).
Dr G. Y. KENNEDY, Sheffield (Porphyrins in marine invertebrates).
G. KERKUT, Cambridge (*Astropecten*; behaviour of gastropods).
Dr R. D. KEYNES, Cambridge (Giant nerve fibres in *Loligo*; electric organs in rays).
Miss L. LAKE, London (Ecology of buoys in Plymouth Sound).
Dr MARIE V. LEBOUR, Cawsand (Decapod crustaceans).
Prof. O. E. LOWENSTEIN, Birmingham (Electrophysiology of elasmobranch labyrinth).
Dr A. G. LOWNDES, Peterborough (Density of marine organisms; Entomostraca).
D. C. H. MCDONALD, London (Architecture of marine laboratories).
D. A. MCGILL, Bucknell, Pa., U.S.A. (Fulbright Scholar) (Iodine metabolism in *Ciona*).
Prof. S. A. MATTHEWS, Williamstown, Mass., U.S.A. (Endocrinology of fish).
Dr A. J. MATTY, Nottingham (Elasmobranch endocrinology).
R. B. MAYNE, Plymouth (Library).
Dr J. E. MORTON, London (Tunicate digestion; pteropod morphology).
Miss M. P. MOSS, Oxford (General).
I. S. R. MUNRO, C.S.I.R.O. Australia (General).
Dr E. NIZET, Liège (Physiology of kidney in *Lophius*).
Miss M. OLIVER, Oxford (General).
A. H. PAPWORTH, Northampton (Plankton).
Miss J. PELLY, Oxford (General).
E. J. PERKINS, London (pH of seashore soils).
D. G. POLLARD, Plymouth (Library).
Dr H. H. POOLE, Dublin (Photoelectric measurement of light).
S. RANASINGHE, Reading (Fauna of cast seaweed).
Dr E. RASMUSSEN, Copenhagen (*Polydora hoplura*).
Dr W. J. REES, British Museum (Natural History) (Library).
Miss B. RICKARD, Plymouth (Library).
Dr J. P. RILEY, Liverpool (Chemistry of sea water).
G. S. SAETERSDAL, Bergen (General).
Dr C. P. SPENCER, Bangor (Physiology of diatoms).
Miss F. A. STANBURY, Plymouth (*Cladophora*).
J. H. STEELE, Aberdeen (Chemistry of Sea Water).
F. C. STOTT, Kingston (Library).
Dr MURIEL F. SUTTON, London (Regeneration in *Ciona*; feeding in *Terebella*).
H. WILSON THOMAS, Plymouth (Library).
Prof. E. B. VERNEY, F.R.S., Cambridge (Physiology of kidney in *Lophius*).
Miss M. WAGSTAFF, Horsham (Movements of *Patella*).
Dr G. P. WELLS, London (*Arenicola*).
Dr MARY WHITEAR, London (Histology of fish skin).
Miss E. WHITEHEAD, London (Biology of *Lasaea rubra*).
J. H. WICKSTEAD, Colonial Fisheries (General plankton).
Dr D. R. WILKIE, London (Dynamic constants of *Raia* muscle).
Miss L. WILSENS, Liège (Physiology of kidney in *Lophius*).
R. F. WRIGHTON, Birmingham (General).
H. V. WYATT, London (Life history of *Calyptraea*).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Dr L. R. Donaldson, Seattle; Dr A. Stephanides, Athens; Dr F. C. Withler, Nanaimo; Dr E. Dahl, Oslo; Dr Y. R. Desai, India; Prof. Irving M. Klotz, Woods Hole; Dr Anton Fr. Bruun, Dr H. Lemche, Torben L. Wolff and F. Jensenius Madsen, Copenhagen; A. E. Hefford, New Zealand; Dr Lois E. TeWinkel, U.S.A.; Dr Olaf Elofson, Uppsala; D. L. Belding, Woods Hole; Dr E. C. T. Holsinger, Ceylon; R. Knapp-Fisher, Newfoundland; A. Richards, La Jolla; Dr A. von Brandt, Hamburg; Dr H. Mann, Hamburg; Dr H. Mertens, Bonn; Dr H. Wiehr, Hamburg; F. W. Moorhouse, South Australia; Prof. S. Stankovic, Belgrade; D. W. le Mare, Penang; Prof. H. Etcheverry and Prof. Regina Cubillos, Valparaiso.

The Royal Danish Research Ship *Galathea* arrived at Plymouth on 20 June 1952, being the last port of call before returning to Copenhagen after her voyage round the world under the scientific leadership of Dr Anton Fr. Bruun.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty-four students from the following Universities and University Colleges: Oxford, Cambridge, Glasgow, Edinburgh, London, Leeds, Sheffield, Nottingham, Newcastle, Southampton, Aberystwyth, Cardiff, Exeter, and from Plymouth Technical College.

Also during the Easter Vacation Mrs F. B. J. Sewell brought a party of five girls from Southampton Grammar School and Eltham High School, Mr I. F. Thomas four boys from Oundle, and Mr R. J. Jones twelve boys from Whitgift School.

Scientific Work of the Plymouth Laboratory Staff

During the year the scope of the work of a number of members of the staff has been much extended by opportunities to take part in the research cruises of R.R.S. *Discovery II*. The Council wish to record their thanks to the Director of the National Institute of Oceanography and his staff for this valuable co-operation, and for the assistance they have given in the work at sea.

In a number of ways also there has been collaboration and co-operation with the Fisheries Laboratories of the Ministry of Agriculture and Fisheries at Lowestoft and of the Scottish Home Department at Aberdeen.

Physics and Chemistry of Sea Water

In conjunction with Dr H. H. Poole, who again visited the laboratory after a long interval, Dr W. R. G. Atkins has worked at a modification of the 'balance by depth' method of measuring transparency of sea water which appears to have certain advantages, though the apparatus is more expensive. A copy of the original apparatus was supplied, from London, to a French laboratory.

Now that their joint work on the scattering of light in sea water has been published in the *Proceedings of the Royal Society* (B, Vol. 140), they are working up further early post-war measurements with the cube photometer to compare the clarity of the water with that obtaining before the war and to study the angular distribution of the light. Dr Atkins has made such a comparison of the phosphate and silicate contents of the water at Station E1, comparing the years 1923, 1925 and 1948, using precisely the same methods of analysis. The winter phosphate maximum in 1948 was 25% below that for 1923, but owing to better utilization the minimum value for the production (minimum because of the possibility of some phosphate being used more than once) was only 16% below 1923. Very curiously the winter silicate maximum in 1948 was also almost exactly 25% below that of 1923.

The seasonal variation in the copper content of sea water was also studied, mainly for 1948. A winter maximum of about 25 mg./m.^{3} fell to an autumn minimum of under 2 mg./m.^{3} for surface water. In April a surface 8 mg./m.^{3} was accompanied by 18 mg. at 50 m., a reduction comparable to that for phosphate, and, like it, due to utilization by the phytoplankton. Papers on the phosphate, silicate and copper have been published in Volume XXXI, No. 3, of the *Journal*.

Dr L. H. N. Cooper has continued to seek an understanding of the richness of the English Channel in plant nutrients and the associated rich production of animals which occurred during the 1920's. In the Celtic Sea in 1950 productive waters containing *Sagitta elegans* and associated biological indicators and reasonably rich in nutrients were found. It is likely that these waters owe their enrichment, at least in part, to regeneration from muds and oozes on the sea bed. Nowhere was there water as rich as that found in the English Channel a quarter of a century ago by Dr Atkins. Large areas of the Celtic Sea were definitely poor in nutrients and plankton.

Since it seemed that the answer was not to be found in some vagary of the circulation of the Celtic Sea, attention was switched to the continental slope. Ways in which deeper richer water may be raised to the surface were examined (see Volume XXX, No. 3, of the *Journal*). Again, no explanation is to be seen in terms of events confined to the continental slope and neighbouring waters. To-day sufficiently rich water in the deeper Atlantic is not within reach of the forces which could bring it up to the surface.

Further evidence accrued from a chance investigation. At the end of October 1951, large numbers of boar-fish, *Capros aper*, were taken by the Laboratory research vessels for the first time for many years. It has been suggested (see Volume XXXI, No. 2, of the *Journal*) that these were thrown up from a canyon by a 'submarine eagre' on 25 September 1951 and then carried towards the Eddystone by currents.

It would now seem that the known facts can be explained only if in the 1920's much of the water in the eastern North Atlantic between 400 m. depth and the bottom was thrust up bodily by 100 m. or more, so raising the level of nutrients accessible to upwelling and capsizing processes. A hypothetical means of achieving this has been developed which requires that the oxygen content of the bottom water of the Atlantic should have decreased steadily since 1921. A station in the Bay of Biscay worked by the Danish Research Vessel *Dana* in 1922 and 1930 has been twice repeated by R.R.S. *Discovery II* in 1952. Below 3000 m. the oxygen content has fallen from 5·8 ml. in 1922 to 5·4 ml. in 1930 and 4·9 ml. in 1952. The distribution of silicate (see Volume XXX, No. 3, of the *Journal*) in the Atlantic bottom water and in the Norwegian and Greenland Seas, if it can be rightly interpreted, holds promise of clarifying the problem.

Dr Cooper and Mr F. A. J. Armstrong spent three weeks on R.R.S. *Discovery II* in the North Atlantic in June and Mr Armstrong a week in September. Observations were made of the distribution of temperature, salinity, inorganic and total phosphorus, silicate and aluminium at four stations, one down to 6000 m. in a deep basin. Mr Armstrong also determined inorganic suspended matter at several depths. He has, in addition, been able to analyse for silicate samples collected by R.V. *Ernest Holt* from the waters around Bear Island and Greenland. These samples were obtained through the courtesy of Mr A. J. Lee of the Ministry of Agriculture and Fisheries Laboratory, Lowestoft.

Monthly cruises to Station E1, and routine determinations of phosphate, total phosphorus and silicate, have been continued by Mr Armstrong, who has also determined and partially analysed the inorganic matter in suspension at various depths at this station throughout a year. The amount found at the surface has been consistently larger than that in deeper water, and there was a decrease at most depths during the summer months.

A spectrophotometric method of determining aluminium in solution in sea water has been studied, to help in estimating that part of the silicate in sea water which may be derived from solution of aluminosilicates.

In January 1952 Mr Armstrong took another series of samples in the Teign Estuary and analysed them for the late Prof. J. H. Orton. Technical and chemical assistance has been given by him to Dr D. P. Wilson for his investigations into the biological differences between sea waters.

Plankton

Dr W. R. G. Atkins and Miss P. G. Jenkins have followed the phytoplankton through a complete year by spectrophotometric measurements of chlorophyll and by identification of the organisms each month. The water column, 0–70 m. at Station E1, had over 1·0 g./m.^{2} of plankton, wet weight, in March and September, the April maximum and the June minimum being 1·32 and 0·15 g./m.^{2}. A comparison with the production obtained from phosphate analyses suggests that the phytoplankton crop each month is rapidly devoured. Identifications were effected by adding nutrient salts to samples of the water and allowing the plants to multiply in diffuse light. By this means the common diatoms were found, as well as seven Chlorophyceae not recorded in previous plankton studies of the region. Chrysophyceae and Dinophyceae were also found, and, of the Cryptophyceae, *Hemiselmis rufescens* Parke appeared in almost pure growth in a 50 m. sample taken in January. The results of this work have been published in Volume XXXI, No. 3, of the *Journal*.

Dr H. W. Harvey has continued investigation of the uptake of inorganic nitrogen compounds by the diatom *Nitzschia closterium*, and the synthesis of pigments in the dark by nitrogen-deficient cells when supplied with a nitrogen source. The uptake of organic phosphorus compounds—inositol hexaphosphate and glycerophosphoric acid—by phosphate-deficient cells in the dark has also been examined. Accounts of these investigations are being published in Volume XXXI, No. 3, of the *Journal*. An account of the technique used for microdetermination of organic phosphorus is in press in the *Analyst*.

A technique is being sought to examine the behaviour of a marine *Chlamydomonas* which is attracted by and swims into a concentration gradient of a number of compounds. It is hoped that such observations may throw light upon the nutritional requirements of this alga, if it proves possible to disentangle the effects of different compounds. Observations at present indicate that these flagellates swim into very low concentrations of ammonium if they are growing in nitrate (when growing in a mixture of ammonium and nitrate they absorb and utilize ammonium in preference to nitrate); into concentrations of manganese when growing in nitrate, but not when supplied with ammonium (a part played by manganese in plant metabolism is stated to lie in the process of utilization of nitrate); into low concentrations of iron; and into a higher partial pressure of carbon dioxide.

The collection of species-pure cultures of marine plant organisms has been maintained throughout the year by Dr Mary Parke, and a number of plant forms not already in the collection have been isolated either from sea-water samples or from mixed cultures. In addition to plant forms, a ciliate, *Uronema schewiakoff*, has been isolated in species-pure culture and has, so far, been kept successfully. The study of new members of the Chrysophyceae with three flagella has been continued.

Owing to the illness of her technical assistant, the maintenance of this collection of marine forms has taken up a great deal of Dr Parke's time this year.

During the year cultures for research purposes have been sent abroad and also to institutions in this country. A great number have also been produced for the use of research workers in the laboratory.

Work has also continued on the compilation of the preliminary Check List of the British Marine Algae.

Miss D. Ballantine, holding a Development Commission Fisheries Research Training Grant, has continued the comparison of methods for the estimation of numbers of nanoplankton organisms other than diatoms in sea water, and this investigation is now approaching completion. It is evident that, of the methods employed, centrifuging and filtering through a collodion membrane give strictly comparable results. The serial dilution technique gives very variable and usually lower results than the above two methods of counting.

Miss Ballantine has carried out some preliminary experiments on the use of penicillin and streptomycin (C. P. Spencer's technique) to isolate pure cultures of naked flagellates, but to date no success has been achieved.

Mr F. S. Russell has given much time to the correction of the proofs of the monograph on the Medusae of the British Isles. In this he has received critical assistance from Dr P. L. Kramp, of the Zoological Museum of the University of Copenhagen, and from Dr W. J. Rees, of the British Museum (Natural History). Dr J. S. Alexandrowicz has given much help in checking the bibliography. The final proofs have now been returned to the printers.

During 1952, Mr P. G. Corbin has observed no change from the prevailing low level of macroplankton production off Plymouth as sampled by half-hour oblique hauls with the 2 m. stramin ring-trawl.

Dr D. P. Wilson, in collaboration with Mr F. A. J. Armstrong, has made some further experiments on biological differences between natural sea waters. As it was not possible this year to obtain water from the Celtic Sea, water from the Firth of Clyde was substituted. This was possible by the kind co-operation of the Scottish Marine Biological Association's staff at Millport. Water collected in the Clyde was sent to Plymouth by rail, and in one experiment yielded better cultures of *Echinus esculentus* larvae than did water from the English Channel. In a mixture of equal volumes of the two waters the larvae did almost as well as in the Clyde water alone. It is interesting to note that both the Celtic Sea and the Firth of Clyde are characterized by a *Sagitta elegans* plankton, the English Channel at the present time being characterized by a *S. setosa* community. In another experiment it was shown that hydrogen-ion concentrations within the range likely for natural sea water have little or no effect on the growth and form of early *Echinus* larvae. Both Clyde and Channel waters, after passing through active carbon, gave poor, abnormal cultures of the larvae. From the carbon through which the waters had been passed weighable amounts of unidentified materials were extracted with acetone; these materials, added to waters of both kinds, resulted in the early death of developing *Echinus* eggs, whereas the control blank extracts from the carbon did not. These results have been published in Volume XXXI, No. 2, of the *Journal*.

Fauna and Flora of the Sea Floor

Dr D. P. Wilson has continued his work on the settlement of *Ophelia bicornis* larvae and considerable progress has been made. The memoir mentioned last year has been published in the *Annales de l'Institut Océanographique*, and since then another paper based on experiments during the summer of 1951 has been prepared for publication in Volume XXXI, No. 3, of the *Journal*. In this paper it is shown that sands may be classed as attractive, neutral or repellent, and that there is considerable variation in the extent to which they are attractive or repellent. Further experiments during the summer of 1952 gave confirmatory evidence in support of this classification, and, in addition, an attractive factor was transferred to acid-cleaned sands, which are normally neutral, and to a purely artificial sand of fused alumina. Similar transferences of a repellent factor were also obtained, though less markedly than for the attractive factor. It was shown that the grade of a sand does influence settlement and metamorphosis to some extent. The results of the latest experiments indicate that organic material, living or dead, on the sand grains plays an important role in inducing or discouraging settlement.

With a view to bringing up to date our knowledge of the Isopoda and Tanaidacea of the Plymouth area, Mr G. M. Spooner has re-examined all available material, preserved and freshly collected. The identity of a number of species has been cleared up. Among additions to the Plymouth fauna is *Anilocra physodes* (L.), first observed in 1951, a warm-water species apparently spreading northward. *Limnoria* is represented by two species, not one only, of which *L. quadripunctata* Holthuis is distinctly more widely spread and abundant than the true *L. lignorum* (Rathke). Observations have been made on the relative distribution of the various *Idotea* species, of which *I. metallica*, an open-water form, is new to the area.

During a month spent at Monaco in June Mr Spooner was able to investigate all obtainable Amphipoda and Isopoda, for a comparison of Mediterranean species with those of Britain. Special attention was given to the genera *Gammarus* and *Jassa*. The dominant marine *Gammarus* of Mediterranean waters, hitherto assumed to be *G. locusta* (L.), is not this but a related species already known from southern England and Belgium which is yet to be described.

Continued work on *Jassa* has largely confirmed the view that more than one distinct species has been confused under the name of *J. falcata* (Mont.), contrary to the views of Sexton & Reid. There are at least three in Britain, and evidently others in the colder antarctic waters. *J. falcata* (Mont.) *sens. str.* is known only from Europe, and may be the only form in more northern parts. It is especially associated with growths of *Tubularia*, though not confined to that habitat. *Jassa marmorata* Holmes is abundant amongst weeds and ascidians on buoys and rafts, and appears to be the characteristic American species, from which area it may have originally spread. *Jassa valida* Dana has been newly recognized from Brixham and Monaco Harbours. Its home apparently is the temperate zone of the southern hemisphere. It is supposed that these three species have arisen under geographical isolation and have been brought together (as in Britain) prematurely by transportation on ships.

Dr H. G. Vevers has published a second paper on the photography of the sea floor in Volume XXXI, No. 2, of the *Journal*. It has now been found that the large aggregations of the brittle-star *Ophiothrix fragilis* previously photographed have been remarkably constant in distribution and density over a period of more than two years. An additional area with a very dense population of the same brittle-star has been photographed near the Eddystone Reef and has been shown to coincide with an area from which Dr E. J. Allen dredged enormous hauls of brittle-stars more than 50 years ago.

A study of variation in the sex ratio of *Asterias rubens* populations has been published in Volume XXXI, No. 1, of the *Journal*, and, in collaboration with Mr James Fisher, a paper on the present population of the North Atlantic gannet (*Sula bassana*) has been published in the *Proceedings of the Xth International Ornithological Congress at Uppsala, 1950*.

Dr Vevers has also continued his work on the carotenoid pigmentation of echinoderms and crustaceans, and, in collaboration with Dr G. Y. Kennedy, of the University of Sheffield, on the isolation and estimation of protoporphyrin in the integument of *Asterias rubens*.

Mr N. A. Holme has nearly completed a paper on the biomass of the bottom-fauna at Plymouth. This will provide a basis for following changes in the productivity and faunistic composition of the benthos in the area.

In continuation of a study of the distribution of certain lamellibranchs, particularly *Ensis*, in relation to grade of the substratum, he visited Tresco in the Scilly Isles in March 1952. The beaches are mostly of a coarse sand derived from the granite rocks of which the islands are formed, and characteristic species of the *Spatangus-Venus fasciata* community were found. The fauna is quite rich and varied, and some species seemed to attain a greater size than in the English Channel.

In September Mr Holme spent a week shore-collecting from the Marine Biological Station at Roscoff, the main object being to trace the southward distribution of *Ensis siliqua*. This species is rare or absent in Jersey, where it is replaced by *Solen marginatus* on open sandy beaches. At Roscoff, *Ensis siliqua* is not very common, and there is reason to believe that it is here close to the southern end of its range. While in France he made a short visit to the Marine Laboratory at Dinard.

In July and August 1952 Mr Holme spent three weeks at sea on R.R.S. *Discovery II* in the North Atlantic, and worked fourteen stations with the bottom-sampler in depths down to 70 fathoms, mainly in the Celtic Sea area. These preliminary samples of the bottom fauna will be of value when it becomes possible to sample in the area with the Association's new ship.

Mr G. R. Forster has designed and developed a dredge which can collect some of the deeper-burrowing animals, such as *Upogebia* and *Callianassa*.

The first model, made in the laboratory from scrap material, has been worked satisfactorily from the *Gammarus* and the *Sula*. An account of this dredge is being prepared for publication, and plans have been drawn up of a stronger and slightly larger model.

With the aid of a Royal Society grant, a Siebe-Gorman compressed-air self-contained breathing apparatus has been purchased for Mr Forster. A considerable amount of diving has been undertaken by him, but he has not yet been able to explore below the *Laminaria* belt outside the Sound, as it extends to depths of 9 or 10 fathoms. With further practice there should be little difficulty in reaching the 'sub-*Laminaria*' zone. A few underwater observations have been made on different types of dredge to see how deeply they dig into the sea bed.

Observations on the migratory behaviour of *Leander* have been continued.

Mr Forster has also made a study of membranes in the gut of decapods, especially *Leander* and *Hippolyte*. These membranes, which are chitinous, were first noticed on the faecal pellets. They may not be true peritrophic membranes as they are probably secreted largely in the posterior end of the pyloric filter press, which is part of the fore-gut.

Comparative studies on marine furcocercous cercariae are being made by Mr R. F. Hutton, working on a Fulbright Research Scholarship. To date, four new species have been isolated from the Plymouth area. The description of one of these species has been published in Volume XXXI, No. 2, of the *Journal*, and another in Volume XXXI, No. 3.

Mr D. B. Carlisle has continued his studies on the Plymouth tunicates, and two papers on the larva of *Polyclinum* and on the British species of *Trididemnum* have been published in Volume XXXI, Nos. 2 and 3, of the *Journal*.

Mr P. G. Corbin's re-examination of the sessile Scyphomedusae (Lucernariidae) has revealed the presence of five species in the Plymouth area. *Haliclystus auricula* is common from time to time; the remaining four species are uncommon. They are *Lucernaria campanulata*, to which *L. discoidea* of Eales is undoubtedly referable; a *Lucernaria* sp., formerly frequently identified as *L. campanulata* but which appears to be undescribed; *Craterolophus tethys*, previously unrecorded; and *Depastrum cyathiforme*, recorded from the area but not recently taken.

Physiology of Marine Animals

Investigations of the physiology of luminescence in marine animals have been continued by Dr J. A. C. Nicol. With the aid of photoelectric recording further information has been obtained about the luminescent responses of *Chaetopterus*. Interest has centred on nervous control of the luminescent cells. It has been found that a single electric stimulus is effective, but an increase in the intensity of the light emitted can be secured by using bursts of stimuli, or by raising the frequency of stimulation. These effects are ascribed to a process of summation in the contractile elements of the glandular cells responsible for discharging the luminescent secretion, and the theoretical implications are discussed in an account which has appeared in Volume XXXI, No. 1, of the *Journal*.

In contrast to *Chaetopterus*, the polynoid worms show intracellular luminescence, and this condition has facilitated a more exact analysis of the processes of nervous regulation. There are six or seven polynoid worms in the Plymouth fauna that emit luminous flashes from their elytra when disturbed, and oscilloscope recordings have been obtained of the luminescent flashes of these animals. It has been found that the normal response is a series of flashes to each stimulus. By regulation of the experimental conditions it has been possible to investigate the interplay of summation, facilitation, and fatigue in the luminescent responses. Part of this work is ready for publication, and the studies are being continued.

Dr Nicol has also been able to extend his investigations to luminescent pelagic species on board R.R.S. *Discovery II* during her third cruise in 1952. Interesting records were obtained of luminescence in *Beroë*, *Pelagia* and *Pyrosoma*, and much material collected for later examination.

Dr J. S. Alexandrowicz has completed his study of the muscle receptors in the thorax of *Homarus vulgaris* and *Palinurus vulgaris*. A paper recording the results of this research has been published in the *Quarterly Journal of Microscopical Science*. Further work on the muscle receptors in crustaceans has revealed that hermit crabs have receptor organs in the abdominal segments situated in the layer of the extensor muscles. These results were published in Volume XXXI, No. 2, of the *Journal*.

The study of the innervation of the heart in *Ligia oceanica* has resulted in establishing the presence of nerve elements arranged on the same lines as in the Decapoda and Stomatopoda. It has also been found that the pericardium is reinforced by alary muscles which have an innervation independent of the heart nerves. The results were published in Volume XXXI, No. 1, of the *Journal*.

Two further parts of the 'Notes on the nervous system of the Stomatopoda' have been prepared for publication. The first of these deals with the system of nervous trunks which run through the thoracic and abdominal segments on the ventral side of the extensor muscles and contain twenty ganglion cells on each side. The nerves arising from these trunks end in the five abdominal segments in neuropile-like networks situated in the pericardial cavity. It is suggested that these nerves have a neurosecretory function. The second contains some observations on small nerve cells with long processes situated in the trunks of the motor nerves. The results have been published in Volume XXIV of *Pubblicazioni della Stazione Zoologica di Napoli*.

Extending these investigations of the nerve elements in the pericardium to decapod crustaceans, Dr Alexandrowicz has found that in all the species investigated (several crabs, *Eupagurus bernhardus*, *Leander serratus*, *Homarus vulgaris*) there are nervous trunks suspended in the pericardial cavity which contain, apart from thicker nerve fibres, a sheath of fine neuropile-like terminations. In some species similar terminations spread also on the pericardium wall. The term 'pericardial organs' has been proposed for these structures, and it is assumed that they are neurosecretory in nature. A paper recording the results of this work has been published in Volume XXXI, No. 3, of the *Journal*.

Dr Alexandrowicz and Mr D. B. Carlisle have collaborated in an investigation of the function of these pericardial organs in crustaceans. It has been shown that extracts from the organs are active in the control of heart beat, and that there are probably two hormones present, one of which gives the fluorescence test for adrenaline.

Mr Carlisle has also investigated the endocrinology of moulting in prawns and crabs. The moult-accelerating hormone, previously found in collaboration with Dr P. Dohrn, in the Mediterranean shrimp *Lysmata* (*Pubb. Zool. Stat. Napoli*, Vol. XXIV), has been found also in *Leander*, but not so far in *Carcinus*. The hormone has been shown to be present in extracts of the cerebral and thoracic ganglia and of the eye-stalks, but not in extracts of muscle or legs. An attempt has been made to isolate the hormone which appears, on the evidence available so far, to be a polypeptide. For the purpose a paper electrophoresis apparatus has been constructed. Further work on this hormone in *Lysmata* was performed in Naples during May 1952.

Dr J. Lowy has completed his work on contraction and relaxation in the adductor muscles of *Mytilus edulis*. It was found that nerve elements located in the visceral ganglia control the speed of relaxation as well as the maintenance of tonic contraction. During normal tonus electrical activity in the muscles consists of intermittent excitatory volleys; muscle potentials also occur in the intervals between volleys. Prolonged tonus is accompanied by a decrease in frequency and amplitude of the excitatory volleys. In connexion with this work it was necessary to develop a more sensitive recording technique, and this was accomplished by adapting a two-channel ink-writing oscillograph. An electrode system was devised which allows the recording of electrical activity of muscle in sea water without artifacts.

In experiments with preparations of the posterior adductor muscle it has been shown that a state of tonus can be abolished by stimulating the visceral ganglia (or the cerebral ganglia, or the sense organs situated along the mantle edge) at an intensity greater than that required for excitation. This causes inhibition of the electrical response in the muscle, followed by relaxation. However, in the intact animal there is often an increase in the frequency of muscle potentials during relaxation after prolonged tonic contraction. This observation is difficult to explain and more experimental work with isolated preparations is needed before a satisfactory hypothesis of the mechanism of relaxation can be formulated. An account of this work will appear in the *Journal of Physiology*.

In continuation of work on the energetics of isolated muscle begun at University College, London, Mr B. C. Abbott is investigating the mechanical, electrical and thermal properties of *Mytilus* retractor muscles in collaboration with Dr Lowy.

Mr Abbott has also started investigations on the uptake of radioactive isotopes of certain elements by unicellular algae.

Ion exchanges in the byssus retractor muscles of *Mytilus* are also being studied.

Fish and Fisheries

Dr G. A. Steven has continued his observations on the Newlyn (Cornwall) spring mackerel fishery with a view to forecasting fishing prospects. A moderately successful season was predicted for this year's fishery. In spite of a very poor start which worried the trade for a time, the total yield was finally in reasonable agreement with expectation. The indications are that next season's fishery will also be a moderately successful one; but, as pointed out in last year's report, the Newlyn mackerel fishery is so greatly influenced by marketing conditions that the fishing yield may bear little relation to actual fish stocks. Occasional divergences from prediction must, therefore, be expected even if the predictions are basically sound.

The trawling survey of grounds in the vicinity of Plymouth for comparison with similar surveys made in 1913–14 and in the early 1920's has been continued. The preliminary trends reported last year continue, i.e. that present catches are on the whole poorer than those of the 1920's but better than those of the 1913–14 period. Much work yet remains to be done, however, before really conclusive results can be presented.

In connexion with the development of the large mid-water net, Mr Steven has been experimenting with a simple type of depressor which can be used and stored as simply as a trawl board. Promising results are being obtained.

Mr P. G. Corbin has obtained further collections of sand-eels (Ammodytidae) from off-shore during trawling with a small-mesh cover on the cod-end of the trawl.

This year Mr Corbin had an opportunity of carrying out open-ocean trials aboard R.R.S. *Discovery II* of the 30 ft. diameter mid-water net referred to in last year's Report of the Council. Several oblique hauls were made with the net; it operated satisfactorily and can be shot and hauled inboard easily and quickly without requiring special tackle or extensive deck space, and when not in use it stows compactly ($1\frac{1}{2} \times 2\frac{1}{2}$ yards). The strain exerted by a net of this size on a fairly long warp (1000–1500 m.) is considerable and presents the major obstacle to towing at speeds fast enough to catch rapidly moving larger creatures. Further information on this problem is being sought.

The preservation of fishing nets, trawl twines and ropes was further studied by Dr W. R. G. Atkins and Mr F. J. Warren, attention being paid to the water absorption. The preservatives tried, save tar on coir, add little weight to the wet ropes because less water is taken up. Care was taken to dry the treated specimens to constant weight before immersion. For use in the sea a longer immersion period than that in the British Standards Institution specification seems desirable. On thin sisal ropes six copper preparations are still under test. These results were published in Volume XXXI, No. 3, of the *Journal*.

Library

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

Dr Mavis Gunther has generously presented to the Library a collection of reprints relating to marine biology and oceanography which belonged to the late Dr E. R. Gunther.

The Library has again been much used by visiting members of the Association.

Published Memoirs

Volume XXXI, No. 1, of the *Journal* was published in June 1952, No. 2 in October 1952, and No. 3 in February 1953.

The following papers, the outcome of work done at the Plymouth Laboratory, have been published elsewhere than in the *Journal* of the Association:

ABBOTT, B. C. & AUBERT, X. M., 1952. The force exerted by active striated muscle during and after change of length. *Journ. Physiol.*, Vol. 117, pp. 77–86.

ALEXANDROWICZ, J. S., 1952. Notes on the nervous system in the Stomatopoda. I. The system of median connectives. *Pubbl. Staz. Zool. Napoli*, Vol. 23, pp. 201–14.

ALEXANDROWICZ, J. S., 1952. Receptor elements in the thoracic muscles of *Homarus vulgaris* and *Palinurus vulgaris*. *Quart. Journ. Micr. Sci.*, Vol. 93, pp. 315–46.

ALEXANDROWICZ, J. S., 1953. Notes on the nervous system in the Stomatopoda. II. The system of dorsal trunks. III. Small nerve cells in motor nerves. *Pubbl. Staz. Zool. Napoli*, Vol. 24, pp. 29–45.

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Membership of the Association

The total number of members on 31 March 1953 was 709, being 50 more than on 31 March 1952; of these the number of life members was 89 and of annual members 620. The number of Associate members is six, Dr W. T. Calman, F.R.S., having died during the year, and Mr W. H. Searle having been elected.

Gift

The Council wishes to record its thanks to Dr Anna M. Bidder for the gift of two elm seats to be placed in the garden of the Plymouth Laboratory.

Finance

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Private Income. The Council gratefully acknowledges the following generous grants for the year:

From the Fishmongers' Company (£400), the Royal Society (£50), British Association (£50), Physiological Society (£30), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s*.), Leeds (£20), Durham (£10. 10*s.*), Manchester (£10. 10*s.*), Sheffield (£10. 10*s.*), Nottingham (£10. 10*s.*), Southampton (£15. 15*s.*), Exeter (£10. 10*s.*), Leicester (£10. 10*s.*), Hull (£10. 10*s.*), and the Imperial College of Science and Technology (£10).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1953-54:

President

Prof. James Gray, C.B.E., M.C., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of Iveagh, C.B., C.M.G.
Sir Nicholas E. Waterhouse, K.B.E.
Col. Sir Edward T. Peel, K.B.E., D.S.O., M.C.
G. P. Bidder, Sc.D.
Vice-Admiral Sir John A. Edgell, K.B.E., C.B., F.R.S.
Prof. A. V. Hill, C.H., O.B.E., Sc.D., F.R.S.
E. S. Russell, O.B.E., D.Sc.
Sir Edward J. Salisbury, Kt., C.B.E., D.Sc., Sec.R.S.
Admiral Sir Aubrey C. H. Smith, K.B.E., C.B., M.V.O.
A. T. A. Dobson, C.B., C.V.O., C.B.E.
Major E. G. Christie-Miller
Morley H. Neale, C.B.E.
The Rt. Hon. Major Sir Thomas L. Dugdale, Bt., M.P.
The Earl of Verulam

COUNCIL

To retire in 1954

J. S. Colman
H. Cary Gilson
Prof. Alastair Graham, D.Sc.
N. A. Mackintosh, C.B.E., D.Sc.
Prof. J. Z. Young, F.R.S.

To retire in 1955

Prof. H. Graham Cannon, Sc.D., F.R.S.
O. D. Hunt
Prof. O. E. Lowenstein, D.Sc.
G. P. Wells, Sc.D.
R. S. Wimpenny

To retire in 1956

J. N. Carruthers, D.Sc.
Prof. H. Munro Fox, F.R.S.
Prof. A. L. Hodgkin, F.R.S.
Prof. J. E. Smith, Sc.D.
Prof. V. C. Wynne-Edwards

Hon. Treasurer

Major E. G. Christie-Miller, 38 Hyde Park Street, London, W. 2

Secretary

F. S. Russell, D.S.C., D.F.C., F.R.S., The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

G. P. Bidder, Sc.D.
R. G. R. Wall (Ministry of Agriculture and Fisheries)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Major E. G. Christie-Miller
  Harrison S. Edwards
Prof. A. C. Hardy, D.Sc., F.R.S. (Oxford University)
S. Smith, Ph.D. (Cambridge University)
Edward Hindle, Sc.D., F.R.S. (British Association)
H. W. Parker, D.Sc. (Zoological Society)
Prof. A. V. Hill, C.H., O.B.E., Sc.D., F.R.S. (Royal Society)

BALANCE SHEET 1952–53

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31st March 1953

	£ s. d.	£ s. d.	£ s. d.
Capital Reserve Account:			
As at 31st March 1952		38,903 17 6	
Add: Grants received and receivable during the year from the Development Fund in respect of cost to date of the New Research Vessel		72,194 18 4	111,098 15 10
Surplus Account:			
As at 31st March 1952		8,234 6 10	
Add: Excess of Income over Expenditure for the year ...		1,546 17 7	
			9,781 4 5
			120,880 0 3
Aquarium Sinking Fund:			
As at 31st March 1952		251 19 10	
Add: Donations for Rebuilding Aquarium Tanks		27 4 8	279 4 6
E. T. Browne Bequest Funds:			
Library Fund as at 31st March 1952	1,224 17 1		
Add: Interest on Investments ...	35 14 3	1,260 11 4	
Special Apparatus Fund as at 31st March 1952	2,633 2 6		
Add: Interest on Investments ...	80 14 10	2,713 17 4	
Scientific Publications Fund as at 31st March 1952	2,229 17 5		
Add: Interest on Investments ...	60 11 9	2,290 9 2	6,264 17 10
Royal Society Grant—Publication of Monograph on Medusae of the British Isles:			
As at 31st March 1952		752 15 8	
Add: Bank Deposit Interest credited during year		13 5 10	766 1 6
Composition Fees Fund:			
As at 31st March 1952		919 18 2	
Add: Fees Received during the year		94 10 0	1,014 8 2

	£ s. d.	£ s. d.	£ s. d.
Fixed Assets:			
At valuations as estimated by the Director at 31st March 1953:			
Boats and Equipment:			
F.V. 'Sula'		11,000 0 0	
Motor Boat 'Gammarus'		200 0 0	
Nets, Gear and General Equipment		100 0 0	
		11,300 0 0	
Laboratory apparatus, equipment and machinery		9,907 19 1	
Library at valuation of Mr Ridgill Trout in January 1941 plus additions at cost		19,487 0 0	
		40,694 19 1	
New Research Vessel (under construction). Cost to 31st March 1953		78,410 7 8	
			119,105 6 9
General Fund Investment, at Book Value £352. 2*s.* 3*d.* 2½ % Treasury Stock ... ... (Market value £208; last year £204)			232 7 10
E. T. Browne Bequest Fund Investments, at cost £5,901. 8*s.* 7*d.* British Transport 3 % Guaranteed Stock 1978/88 ... ... (Market value £4,750; last year £4,544)			5,795 3 6
Composition Fees Fund Investments, at cost:			
£18. 8*s.* 6*d.* 2½ % Treasury Stock		15 15 0	
£1,072. 12*s.* 3*d.* British Transport 3 % Guaranteed Stock 1978/88 ... ... (Market value £874; last year £729)		998 13 2	1,014 8 2
Current Assets:			
Stocks on Hand, as valued by the Director:			
Specimens	600 0 0		
Chemicals	250 0 0		
Journals	400 0 0		
		1,250 0 0	

Buildings Reconstruction Fund:			
As at 31st March 1952		808 7 1	
Add: War Damage Compensation received during the year	1,433 10 5		
Less: Receivable at 31st March 1952	139 14 4		
		1,293 16 1	
		2,102 3 2	
Less: Expenditure during the year		803 8 2	
			1,298 15 0
Radioactive Substances Laboratory Building Fund:			
Grants Received from the Development Fund		559 15 2	
Expenditure during the year...		743 4 10	
Balance *per contra*		183 9 8	
Radioactive Substances Research Fund:			
Grants Received from the Development Fund		876 0 0	
Less: Expenditure during the year		291 16 8	
			584 3 4
Research Fund—Miss D. Ballantine:			
Grants Received from the Development Fund		389 7 6	
Less: Expenditure during the year		302 9 3	
			86 18 3
Current Liabilities:			
Accrued Expenses, etc.		1,113 19 5	
Subscriptions and Grants Received in advance		167 1 10	
Instalments due under contract for construction of New Research Vessel		—	
			1,281 1 3

Note: The Association had a capital commitment at 31st March 1953 in respect of the balance of the cost of the New Research Vessel of £60,670 which will be recoverable under a Development Fund Grant.

£132,455 10 1

Sundry Debtors:			
Sales of Specimens, Journals, Nets and Hydrographical Gear	926 3 1		
Recoverable Expenditure	436 7 5		
		1,362 10 6	
Prepayments		81 7 4	
Radioactive Substances Laboratory Building Fund:			
Balance *per contra*		183 9 8	
Balance at Bank and Cash in Hand:			
Coutts & Co., London	£ s. d		
Current Account...	1,903 15 8		
Deposit Account...	766 1 6		
	2,669 17 2		
Lloyds Bank Ltd., Plymouth ...	699 19 0		
Cash in Hand	61 0 2		
		3,430 16 4	
			6,308 3 10

John E. Harris }
O. D. Hunt } *Members of the Council*

£132,455 10 1

Report of the Auditors to the Members of the Marine Biological Association of the United Kingdom:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion and to the best of our information and according to the explanations given to us, the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31st March 1953, and of the excess of income over expenditure for the year ended on that date.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for our audit. In our opinion the Association has kept proper books of account and the above mentioned accounts, which are in agreement therewith, give in the prescribed manner the information required by the Companies Act, 1948.

Norwich Union House
2, St Andrew's Cross
Plymouth
13th May 1953.

Price Waterhouse & Co.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1953

	£	s.	d.	£	s.	d.
SALARIES, including Pay Additions, and Association's contribution to Superannuation Scheme and National Insurance				22,877	16	4
LABORATORY AND BOATS' CREWS' WAGES, including Pay Additions, National Insurance, contributions to Superannuation Scheme, War Bonus and Employers' Liability Insurance				17,991	18	7
UPKEEP OF LIBRARY				854	19	0
SCIENTIFIC PUBLICATIONS, *less* sales				1,700	9	10
UPKEEP OF LABORATORIES AND AQUARIUM:						
Buildings and Machinery	568	19	11			
Electricity, Gas, Coal and Water	870	5	2			
Chemicals and Apparatus	936	6	9			
Insurances, Ground Rent and Rent of Stores ...	163	10	0			
Travelling Expenses	610	0	6			
Audit Fee	63	0	0			
Stationery, Postages, Telephone, Carriage and Sundries	778	15	5			
Specimens	197	2	8			
				4,188	0	5
MAINTENANCE AND HIRE OF BOATS:						
Petrol, Oil, Paraffin, etc.	679	4	9			
Maintenance of and Repairs to Nets, Gear and Apparatus	2,467	11	3			
Boat Hire, Collecting Expenses and Upkeep of Truck	221	11	9			
Insurances	631	13	4			
Hire of R.V. 'Sabella'	1,380	0	0			
				5,380	1	1
ENTERTAINMENT EXPENSES				59	8	3
BALANCE, being excess of Income over Expenditure for the year				1,546	17	7
				£54,599	11	1

	£	s.	d.	£	s.	d.	£	s.	d.
GRANTS AND TABLE RENTS:									
Ministry of Agriculture and Fisheries grant from Development Fund				47,834	0	0			
Fishmongers' Company				400	0	0			
Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of: London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Southampton £15. 15*s*., Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10. 10*s*., Hull £10. 10*s*., Sheffield £10. 10*s*., Imperial College £10, and Ministry of Works £104)				1,176	2	1			
							49,410	2	1
SUBSCRIPTIONS (excluding those received in advance)							587	4	0
FEES FOR TESTS OF MATERIALS							—		
SALES:									
Specimens				2,432	12	0			
Fish				452	15	2			
	£	s.	d.						
Nets, Gear and Hydrographical Apparatus	870	7	7						
Less: Cost of Materials ...	585	14	11						
				284	12	8			
							3,169	19	10
INTEREST ON INVESTMENTS							37	4	10
INTEREST ON BANK DEPOSITS LESS CHARGES							42	12	7
SALE OF DR M. V. LEBOUR'S BOOK							6	17	6
SALE OF 'PLYMOUTH MARINE FAUNA'							1	7	6
AQUARIUM:									
Admission Fees				1,739	4	10			
Sale of Guides				128	18	6			
				1,868	3	4			
Less: Maintenance of Building	101	17	11						
Printing Guides and Tickets	169	11	8						
Advertising	38	17	0						
Food	107	8	0						
Wages	106	6	0						
				524	0	7			
							1,344	2	9
							£54,599	11	1

INDEX

GENERA AND SPECIES

Abra, 12–47, 91
— *alba*, 12–47, 91
— *nitida*, 42
— *prismatica*, 17–42
Acanthodoris pilosa, 370–82
Acarus basteri, 357
Acholoë astericola, 65–84, 161–72, 274, 284
Acidostoma obesum, 16
Acrocnida brachiata, 17, 31, 35, 195, 282–8
Acteon tornatilis, 17, 37
Alcyonidium, 28, 31
Alcyonium, 23, 29
Aletes minutus, 358
Aloidis, 92, 93, 103
Alpheus ruber, 26
Alysia, 287
Ammotrypane aulogaster, 31
Ampelisca brevicornis, 16, 26
— *diadema*, 21, 25, 31
— *spinipes*, 16–31
— *tenuicornis*, 16–34
Amphicteis gunneri, 16, 29, 31
Amphioxus lanceolatus, 23–44
Amphipholis squamata, 27
Amphiphorus lactifloreus, 543
Amphitrite crinata, 381
— *edwardsi*, 12, 169, 273–83
— *gracilis*, 275–86, 381
— *johnstoni*, 72, 169, 273–86
— *robusta*, 161, 169, 273
Amphiura filiformis, 12–34
Anilocra physodes, 536
Anodonta, 99, 270
— *grandis*, 376
Anomia, 378
Antarctomysis maxima, 437–9
Aphrodite aculeata, 80, 381
Aphya pellucida, 35
Aplidium zostericola, 250
Aplysia punctata, 371, 382
Aporrhais serresiana, 91
Archiascidia neapolitana, 249–65
Archidistoma aggregatum, 249–50
Archidoris britannica, 371
Arctica islandica, 91
Arctonoë fragilis, 161, 170
— *pulchra*, 283
— *vittata*, 283
Arenicola marina, 51–63, 72, 212, 275–84
Arhodeoporus, 362
Arion empiricorum, 235
Arion hortensis, 369–82
Artemia salina, 154–7, 411–47
Ascidia mentula, 17
Ascidiella aspersa, 17, 27, 35
Astacus, 289
Asterias rubens, 14, 111, 164–5, 235–46, 537
Asterina gibbosa, 164–70
Asterionella, 525
Astriclypeus manni, 487
Astropecten, 162–70, 284
— *aurantiacus*, 162
— *irregularis*, 72, 162–170, 282
— *pentacanthus*, 162
Athanas nitescens, 315

Balanoglossus, 32
Balanus balanoides, 107–26, 297–303
— *crenatus*, 107–26, 297–8, 301–4, 472
— *improvisus*, 472
Bathyporeia elegans, 15, 25
— *tenuipes*, 15–33
Beroë, 539
Biddulphia, 151, 428–9, 442
— *regia*, 152, 154
— *sinensis*, 399, 400, 413–14, 447
Bolinopsis, 428
Botryllus, 266
Branchiomma vesiculosum, 271, 365, 544
Brissopsis lyrifera, 91, 477–89

Calanus finmarchicus, 305–13, 412–47
— *simillimus*, 437–9
Calappa marmorata, 441
Callianassa subterranea, 10–29, 537
Callista chione, 42, 195–6
Calyptraea chinensis, 380–3
Cambarus, 289, 290
Cançer irroratus, 175
— *pagurus*, 175, 191
Capros aper, 527, 532
Caranx trachurus, 201
Carcinus maenas, 426–8
Cardium, 93
— *crassum*, 42
— *echinatum*, 15–42
— *edule*, 101
— *ovale*, 42
— *scabrum*, 28, 42
Cellaria, 3–37
Cepola rubescens, 204–7
Cerianthus lloydi, 16–37, 195

Chaetoceros decipiens, 394, 422, 447
— *socialis*, 434
Chaetopterus variopedatus, 16–37, 71–8, 274–5, 538–9, 544
Cheirocratus intermedius, 16
Cheyletus eruditus, 357
— *robertsoni*, 357
Chlamydomonas, 135, 393–447, 534
Chlamys opercularis, 10–42, 244–6
Chlorella stigmatophora, 392–447
Chromulina pleiades, 135, 393, 446
— *pusilla*, 140, 147, 393, 446
Ciona intestinalis, 249–66
Clavelina lepadiformis, 250, 264–5
— *phlegrea*, 264
Coccolithus huxleyi, 393, 446
Cochlodesma praetenue, 15–37
Conilera cylindracea, 28
Copidognathopsis, 360–2
Copidognathus acutus, 360
— *arenarius*, 361
— *curassaviensis*, 360
— *gracilipes*, 359–62
— — *largiforatus*, 361
— — *quadricostatus*, 361
— *granulatus*, 359–64
— *glyptoderma*, 359–64
— *oculatus*, 360–4
— *rhodostigma*, 360–2
— *submarinus*, 361
— *tabellio*, 360
Corbula gibba, 12, 41–2
Cordylophora lacustris, 544
Coryne eximia, 358
Corystes cassivelaunus, 35, 441
Coscinodiscus concinnus, 150–4, 394–447
Cottus bubalis, 207
Crangon vulgaris, 315
Craterolophus tethys, 538
Cryptomya californica, 99
Cucumaria elongata, 21–31
— *lactea*, 34
Cultellus pellucidus, 12–47
Cuspidaria, 103
Cylichna cylindracea, 12–37
Cypridina, 78
Cyprina islandica, 36–45, 78, 91

Daphnia, 315
Dentalium entalis, 39, 47
Depastrum cyathiforme, 538
Diastylis laevis, 21, 26, 31, 33
Diazona violacea, 249–50, 263–5
Dicrateria inornata, 393–446
Diplodonta rotundata, 42
Diplosoma listerianum, 17, 523–4, 543
Ditylum brightwellii, 394–447
Dodecaceria concharum, 381
Donax vittatus, 42, 376
Dosinia lupinus, 15–42
Drepanopus pectinatus, 437, 439
Dromia, 289

Ebalia, 16
— *cranchi*, 31, 33
— *tuberosa*, 28
Echinarachnius brevis, 487
Echinocardium, 15–47, 195–6, 477–89
— *cordatum*, 15–47, 72, 195–6, 477–89
— *flavescens*, 477–89
— *pennatifidum*, 477–88
Echinocyamus pusillus, 17–37, 487
Echinus, 465, 535
Edwardsia, 16–37
Ensis arcuatus, 15, 26, 42
— *ensis*, 26, 35, 42
— *siliqua*, 195–6, 537
Eone, 12
Erichthonius brasiliensis, 16
Eriocheir, 289
Eteone picta, 15
Eucampia zoodiacus, 394–447
Eulima glabra, 12, 33
Eunice harassi, 26–34
Eunoë nodosa, 65, 82
Eupagurus, 34, 540
— *prideauxi*, 14
Eurynome aspera, 34
Eurytemora hirundoides, 413, 447
Eusepia officinalis, 270
Evadne nordmanni, 149
Evarne impar, 65
Evasterias troschelii, 161, 170
Exuviaella baltica, 393–446

Fibularia craniolis, 487
Flabellum variabile, 235
Fucus, 340–4
— *serratus*, 337, 338
Fungia symmetrica, 235

Gadus luscus, 200, 207
— *merlangus*, 207
— *morrhua*, 200, 203
— *pollachius*, 200
Gafrarium minimum, 42
Galathea, 16, 28–34
Gamasus marinus, 357
Gammarus locusta, 536
— sp. allied *locusta*, 536
Gari fervensis, 15–42
— *tellinella*, 28, 42
Gastrosaccus sanctus, 26
Gattyana cirrosa, 65–84, 161–73, 273–84

Gecarcinus, 289
Glossobalanus sarniensis, 17–37
Glossus humanus, 85–105
Glycera, 26–9, 31–5
Glycymeris glycymeris, 42, 86
Goniada, 15, 33
Goniaulax, 403, 435
— *spinifera*, 525
Gonoplax rhomboides, 23–4
Grammatophora marina, 358
Gymnodinium, 135–45, 392–447, 525
— *brevis*, 434

Halacarus ctenopus, 357
— *granulatus*, 357–61
— *oculatus*, 357–60
— *rhodostigma*, 357–63
Halarachne halichoeri, 357
Haliclystus auricula, 538
Halolaelaps glabriusculus, 357
— *marinus*, 357
Halosydna brevisetosa, 161, 169, 273
— *gelatinosa*, 66
Harmothoë, 65–84, 273–88
— *aspera*, 65
— *imbricata*, 65, 72
— *impar*, 65, 69
— *longisetis*, 65
— *lunulata*, 65, 83, 282–6
— *spinifera*, 65–9, 280–5
— *torquata*, 65
Harpinia antennaria, 16, 21
Helix, 270, 491
Hemimysis lamornae, 205, 392–447
Hemioniscus balani, 111, 126
Hemiselmis rufescens, 133–47, 393–446, 534
Henricia sanguinolenta, 164–5
Hesperonoë adventor, 161
Heterocarpa glomerata, 250, 266
Hiatella arctica, 17, 21, 33–7
Hippolyte varians, 315–16, 538
Holothuria forskalii, 544
Homarus, 175–7, 184, 188–91
— *vulgaris*, 175, 191, 521, 539–40, 542
Hyalinoecia bilineata, 27–31
— *tubicola*, 14

Idotea metallica, 536
Isocardia cor, 85–105
Isochrysis galbana, 393, 446

Jasmineira elegans, 27
Jassa, 536
— *falcata*, 536
— *marmorata*, 536
— *valida*, 536

Kelliella, 92

Labidoplax digitata, 17–37, 282
Labrus bergylta, 207
— *mixtus*, 207
Lagisca extenuata, 65–84
Laminaria, 538
Lanice conchilega, 274–83
Lauderia borealis, 394–447
Leander serratus, 289–94, 441, 538, 540
— *squilla*, 289–93, 315, 441
Lepidasthenia argus, 66, 70, 82–3, 161–72, 273–85
— *stylolepsis*, 65, 82–3
Lepidochitona cinereus, 373, 382
Lepidonotus clava, 66–83
— *squamatus*, 65–83
Leptognathus falcatus, 357–63
Lepton squamosum, 28–42
Leptosynapta inhaerens, 23, 33, 72, 282–8
Leucothoë, 16, 25, 35
— *lilljeborgi*, 15, 25
— *spinicarpa*, 15
Licmophora lyngbyei, 394, 447
Ligia oceanica, 539
Lima loscombi, 42
Limnoria lignorum, 536
— *quadripunctata*, 536
Lissoclinum pseudoleptoclinum, 524
Lohmannella falcata, 364
Loligo, 205, 543
Lophius piscatorius, 321–8, 329–36
Lucernaria campanulata, 538
— *discoidea*, 538
Luidia, 162–70
— *ciliaris*, 162, 164
— *fragilissima*, 163
Lumbriconereis, 16–35
Lutraria, 10, 12, 28, 42, 93
— *lutraria*, 41, 42, 195
— *magna*, 28
Lyonsia norwegica, 17, 37
Lysidice ninetta, 274–87
Lysilla loveni, 193
Lysmata seticaudata, 289, 294, 524, 540, 543

Macoma, 20
Mactra, 491, 495
— *corallina*, 15, 26, 42, 43
Maera othonis, 16, 31
Magelona cincta, 16–35
— *papillicornis*, 15–35, 150–3
Maia squinado, 175–91, 294
Malmgrenia castanea, 65–84
Marphysa belli, 29
— *sanguinea*, 280–2
Marthasterias glacialis, 164–5
Massartia rotundata, 393, 447
Meganyctiphanes norwegica, 437, 441

Melinna palmata, 12, 15, 26–9
Metridium senile, 544
Mesodinium, 415
Mesopodopsis slabberi, 408–9, 447
Modiolus, 99
— *phaseolinus*, 27, 42
Montacuta bidentata, 12, 15, 42
— *ferruginosa*, 15–42
Mormoniella, 287
Mugil chelo, 199, 203
Musculus marmoratus, 17, 37, 42
Mya, 491, 495
Myrtea spinifera, 42
Mysella bidentata, 12, 15, 42
Mysia undata, 21, 29, 42
Mytilus californianus, 372, 465–7, 491
— *edulis*, 90, 244–6, 270, 368–82, 449–75, 491–5, 523, 525, 540–1, 543
Myxicola infundibulum, 29, 195, 275, 280, 365, 544

Nassarius, 15
— *reticulatus*, 195
Natica poliana, 15, 26
Navicula, 151
Nebalia bipes, 16
Nematonereis unicornis, 27
Neoamphitrite, 169
Neomysis integer, 408, 447
— *vulgaris*, 395
Nephrops norvegicus, 122
Nephthys, 12, 15, 26–35
Nereis, 24, 35, 205
— *diversicolor*, 24
Nerine, 31, 35
Nitzschia, 151, 394–447
— *closterium*, 394–447, 534
— *integer*, 409
— *minutissima*, 447
— *seriata*, 394, 447
Notomastus latericeus, 12, 29
Nototropis vedlomensis, 15
Nucella lapillus, 111
Nucula hanleyi, 42
— *nitida*, 15
— *nucleus*, 17–42
— *sulcata*, 91
— *turgida*, 17–42
Nyctiphanes, 78

Octopus vulgaris, 270
Oikopleura dioica, 150–60, 428
Onos tricirratus, 204
Onuphis conchylega, 28
Ophelia bicornis, 24, 209–33, 337, 535–6
Ophiothrix fragilis, 17–46, 537
Ophiura affinis, 17–35
Ophiura texturata, 14, 17
Ostrea edulis, 99, 374–7, 465, 491–5
Owenia, 12, 15, 27–35
Oxydromus, 33
Oxyrrhis marina, 393–447

Pachygnathus minutus, 357–9
— *notops*, 357
— *sculptus*, 357
— *seahami*, 357, 362–3
Pagellus centrodontus, 319
Palaemon, 290–4, 315–16, 524
— *elegans*, 293, 315
— *serratus*, 290–3, 315–17.
Palaemonetes, 315
Palinurus vulgaris, 521, 539, 542
Pallasia murata, 30–1
Palmipes membranaceus, 164–5
Pandalina brevirostris, 315
Pandalus montagui, 315
Panulirus, 290, 294
Paphia pullastra, 101
Patella vulgata, 373, 382
Pavlova gyrans, 393, 446
Pecten maximus, 10
Pectinaria, 28
— *auricoma*, 16
— *koreni*, 16–35
Pedalion, 373
Pelagia, 539
Peridinium micans, 401
— *triestinum*, 401
— *triquetum*, 525
— *trochoideum*, 135, 393–447
Perigonimus, 91
Perinereis cultrifera, 381
Petta pusilla, 28
Phacoides borealis, 15–42
Phaeocystis pouchetii, 414, 446
Phascolion strombi, 12–16, 31
Phascolosoma, 28, 282–4
— *elongatum*, 282–3
— *vulgare*, 282
Philine, 12, 26
Philocheras trispinosus, 315
Photis longicaudata, 21, 31
Phyllochaetopterus, 26–35
Phyllodoce, 34
Pisaster, 170
Planktoniella, 524
Platymonas apiculata, 393, 447
Platynereis dumerili, 380–3
Pleuronectes microcephalus, 204
— *platessa*, 149, 319
Pollicipes, 315
Polycarpa pomaria, 31
Polycirrus aurantiacus, 282

Polycirrus caliendrum, 275–86
Polyclinum, 538
Polymnia nebulosa, 72, 274–87
Polynoë aspera, 65
— *cirrata*, 65
— *lunulata*, 65, 68
— *scolopendrina*, 65–84, 273–88
— *squamata*, 65
— *torquata*, 65, 69
Polyprion americanum, 207–8, 527
Polysiphonia ureolata, 203
Porania pulvillus, 14, 164–5
Porcellana longicornis, 16, 35
— *macrocheles*, 412, 441
Porphyra umbilicalis f. *laciniata*, 522, 543
Portunus depurator, 26–8
— *holsatus*, 16
— *marmoreus*, 16
— *puber*, 80, 180–9
Praunus flexuosus, 408–410, 447
— *neglectus*, 397–410, 447
Processa canaliculata, 315
Prorocentrum micans, 393, 446, 525
— *triestinum*, 393, 446
Pseudopedinella, 138, 145, 393, 446
Pseudoprotella phasma, 33, 35
Pterocera, 491
Ptychodera minuta, 282
Pugettia producta, 175
Pyramimonas grossii, 135–45, 393–447
Pyrosoma, 539

Raia clavata, 201, 270
Raphignathus, 357
Rhina squatina, 199–203
Rhizosolenia, 441
Rhodomonas, 396–447
Rhodymenia, 367, 368
Rhombognathopsis, 362
Rhombognathus seahami, 362

Sabella pavonina, 275, 365, 544
Sagitta, 412
— *elegans*, 2, 45, 532, 535
— *setosa*, 2, 45, 535
Scalpellum scalpellum, 26, 31–5
Schizothoerus nuttallii, 103
Sciurus niger, 245
Scoloplos armiger, 31, 33
Scutovertex minutus, 357
— *sculptus*, 357
Sergestes, 78
Sesarma, 289
Sigalion, 15
— *mathildae*, 25, 26, 34
Siniognathus minutus, 364
— *sculptus*, 357–9, 364
Siphonoecetes colletti, 15
Skeletonema costatum, 394–447
Solaster papposus, 164
Solecurtus, 24–30, 93, 101
— *chamasolen*, 23–42, 101
— *scopula*, 28, 42
Solen marginatus, 537
Spatangus purpureus, 28, 72, 477–89, 537
Sphenia binghami, 99
Spirographis, 365
Spirontocaris cranchii, 315
— *pusiola*, 315
Spirorbis borealis, 337–45
Spisula, 12, 15, 41–6
— *elliptica*, 41, 42
— *solida*, 46
— *subtruncata*, 42–4
Squalus acanthias, 45
Squilla mantis, 175–91, 521
Staurocephalus rudolphi, 33
Sthenelais limicola, 35
Stichastrella rosea, 164–5
Stichococcus cylindricus, 394, 447
Stolonica socialis, 250
Sula bassana, 537, 543
Syracosphaera carterae, 401–46

Tellina, 86, 101
— *baltica*, 376
— *crassa*, 23–9, 42, 101
— *donacina*, 31, 34, 42
— *fabula*, 15, 26, 42
— *pygmaea*, 42
— *tenuis*, 44, 90, 99, 376
Temora longicornis, 150–4
Terebella lapidaria, 275, 280, 281
Teredo, 491
Thalassiosira gravida, 394–447
— *nordenskiöldii*, 414, 447
Thracia, 21
— *convexa*, 42
— *pubescens*, 35
— *villosiuscula*, 42
Thyasira flexuosa, 12–42
Thyone fuscus, 31
— *raphanus*, 31
Thysanoessa inermis, 425–47
— *raschii*, 441
Tigriopus fulvus, 413, 447
Torpedo nobiliana, 199–203
— *torpedo*, 199
Trichodesmium, 433
Trididemnum, 538
Trombidium fucicolum, 357
Tubularia, 536
Turritella communis, 12, 14, 46

Uca, 289
Upogebia, 10, 27–39, 205, 537
— *deltaura*, 15, 27–31
— *stellata*, 27
Uraster rubens, 235
Urechis caupo, 161, 374
Uronema schewiakoff, 534
Urothoë elegans, 16, 25
— *grimaldii*, 15, 25
— *inermis*, 25
— *poseidonis*, 15, 25

Venerupis rhomboides, 17, 31, 37, 42
Venus, 1, 14
— *casina*, 42
— *fasciata*, 28, 42, 101, 537
— *gallina*, 32
— *ovata*, 17–42
— *striatula*, 12–42
Verruca stroemia, 297–304
Virgularia mirabilis, 16, 37

Xylophaga, 491

CHECK-LIST OF BRITISH MARINE ALGAE

INDEX TO GENERA

Acinetospora, 502
Acrochaete, 499
Acrochaetium, 508
Acrosiphonia, 499
Acrosorium, 513
Acrothrix, 504
Aglaothamnion, 512
Aglaozonia, 505
Ahnfeltia, 510
Alaria, 506
Amphithrix, 516
Anabaena, 516
Anacystis, 515
Antithamnion, 512
Antithamnionella, 512
Aphanocapsa, 515
Aphanothece, 515
Apistonema, 502
Apoglossum, 513
Areschougia, 507
Arthrocladia, 505
Ascocyclus, 504
Ascophyllum, 506
Asparagopsis, 511
Asperococcus, 505
Asterocytis, 508
Atractophora, 511
Audouinella, 509
Bangia, 508
Battersia, 506
Bifurcaria, 506
Bipedinomonas, 498
Blastophysa, 500
Blidingia, 498
Bolbocoleon, 499
Bonnemaisonia, 511
Bornetia, 512
Bostrychia, 513
Brachiomonas, 498
Brachytrichia, 516
Brongniartella, 513
Bryopsis, 500
Buffhamia, 505
Calliblepharis, 509
Callithamnion, 512
Callocolax, 511
Callophyllis, 511
Calosiphonia, 509
Calothrix, 516
Capsosiphon, 498
Carpomitra, 505
Carteria, 498
Castagnea, 504
Catenella, 509
Ceramium, 512
Chaetobolus, 499
Chaetomorpha, 499
Chaetopteris, 506
Chamaesiphon, 515
Champia, 511
Characium, 498
Chilionema, 502
Chlamydomonas, 498
Chlorella, 498
Chlorochytrium, 498
Chlorogloea, 515
Chloromeson, 501
Chondria, 513
Chondrus, 510
Chorda, 506
Chordaria, 504
Choreocolax, 511
Choreonema, 510
Choristocarpus, 506
Chromulina, 502
Chroococcus, 515
Chrysonema, 502
Chrysotila, 502
Chylocladia, 511
Cladophora, 499
Cladosiphon, 504
Cladostephus, 506
Coccochloris, 517
Codiolum, 498
Codium, 500
Colaconema, 508–9
Colpomenia, 505
Compsonema, 502
Compsothamnion, 512
Conchocelis, 508
Corallina, 510
Cordylecladia, 509
Corynophlaea, 504
Corynospora, 512
Crouania, 512
Cruoria, 509
Cruoriella, 510
Cruoriopsis, 509
Cryptopleura, 513
Ctenosiphonia, 515
Cutleria, 505
Cylindrocarpus, 504
Cystoclonium, 509
Cystoseira, 506
Dasya, 513
Delesseria, 513
Derbesia, 500
Dermocarpa, 515
Desmarestia, 505
Desmotrichum, 505
Dichosporangium, 503
Dichothrix, 516
Dicrateria, 502
Dictyopteris, 506
Dictyosiphon, 505
Dictyota, 506
Dilophus, 506
Dilsea, 510
Diplocolon, 516
Dudresnaya, 510
Dumontia, 510
Dunaliella, 498
Ectocarpus, 503
Ectochaete, 499
Elachista, 504
Endoderma, 499
Endodictyon, 503
Enteromorpha, 498
Entocladia, 499
Entonema, 507
Entophysalis, 516
Epicladia, 499
Epilithon, 510
Erythrocladia, 508
Erythrodermis, 510
Erythroglossum, 513
Erythropeltis, 508
Erythrotrichia, 508
Eudesme, 504
Euthora, 511
Falkenbergia, 511
Feldmannia, 503
Fosliella, 514
Fremyella, 516
Fucus, 506
Furcellaria, 509
Gastroclonium, 511
Gayella, 499
Gelidium, 509
Giffordia, 503
Gigartina, 510
Giraudia, 505
Gloeocapsa, 515
Gloeochrysis, 502
Gloeocystis, 502
Gloeothece, 515
Gloiosiphonia, 510
Gobia, 505
Gomontia, 499
Gonimophyllum, 513
Goniotrichum, 508
Gracilaria, 509
Grateloupia, 511
Griffithsia, 512
Gymnogongrus, 510
Haematocelis, 510
Halarachnion, 509
Halicystis, 500–1
Halidrys, 506
Haloglossum, 508
Halopitys, 513
Halopteris, 506
Halosphaera, 501
Halothrix, 504

Halurus, 512
Halymenia, 511
Haplospora, 505
Harveyella, 511
Hecatonema, 503
Helminthocladia, 509
Helminthora, 509
Herponema, 503
Heteromastix, 498
Heterosiphonia, 513
Hildenbrandia, 510
Himanthalia, 506
Holmsella, 511
Holopedia, 515
Hydrocoleum, 516
Hyella, 516
Hymenoclonium, 511
Hypoglossum, 513
Ilea, 508
Isactis, 516
Isochrysis, 502
Isthmoplea, 504
Jania, 510
Kallymenia, 511
Laminaria, 506
Laminariocolax, 503
Laurencia, 513
Leathesia, 504
Leblondiella, 505
Leptonema, 504
Liebmannia, 504
Lithoderma, 503
Lithophyllum, 510
Lithothamnion, 510
Litosiphon, 505
Lola, 501
Lomentaria, 511
Lophosiphonia, 513
Lyngbya, 516
Mastigocoleus, 516
Melobesia, 511
Membranoptera, 513
Meredithia, 511
Merismopedia, 515
Mesogloia, 504
Mesophyllum, 511
Microcladia, 512
Microchaete, 516
Microcoleus, 517
Microcoryne, 504
Microspongium, 504
Mikrosyphar, 503
Monospora, 512
Monostroma, 498
Myriactis, 504
Myriactula, 504
Myriocladia, 504
Myriogramme, 513
Myrionema, 504
Myriotrichia, 505
Naccaria, 511
Nannochloris, 498
Neevea, 508
Nemalion, 509
Nemastoma, 514
Nematochrysis, 502
Nephrochloris, 501
Nitophyllum, 513
Nodularia, 516
Nostoc, 516
Ochlochaete, 499
Ochromonas, 502
Odonthalia, 513
Olisthodiscus, 501
Oncobyrsa, 516
Oscillatoria, 517
Ostreobium, 500
Padina, 506
Pantoneura, 513
Pavlova, 502
Pelvetia, 506
Percursaria, 498
Petalonia, 505
Petrocelis, 509
Petroderma, 503
Petroglossum, 510
Petrospongium, 504
Peyssonelia, 510
Phaeococcus, 502
Phaeocystis, 502
Phaeophila, 499
Phaeosaccion, 505
Phaeostroma, 503
Phloeospora, 504
Phormidium, 517
Phycodrys, 513
Phyllitis, 505
Phyllophora, 510
Pilinia, 499
Platoma, 509
Platychrysis, 502
Platymonas, 498
Plectonema, 516
Pleonosporium, 512
Pleurocapsa, 516
Plocamium, 509
Plumaria, 512
Polyides, 510
Polyneura, 513
Polysiphonia, 513
Porphyra, 508
Porphyrodiscus, 510
Porphyropsis, 508
Prasinocladus, 498
Prasiola, 499
Pringsheimia, 499
Pringsheimiella, 499
Protoderma, 499
Prymnesium, 502
Pseudanabaena, 516
Pseudolithothamnion, 514
Pseudopedinella, 502
Pseudopringsheimia, 499
Pseudulvella, 499
Pterocladia, 509
Pterosiphonia, 513
Ptilota, 512
Ptilothamnion, 512
Punctaria, 505
Pylaiella, 503
Pyramimonas, 498
Ralfsia, 503
Rhizochloris, 501
Rhizoclonium, 500
Rhizoglossum, 513
Rhodochorton, 509
Rhododermis, 510
Rhododiscus, 510
Rhodomela, 513
Rhodophyllis, 509
Rhodophysema, 510
Rhodymenia, 511
Rivularia, 516
Saccorhiza, 506
Sauvageaugloia, 504
Schizothrix, 517
Schizymenia, 509
Schmitziella, 511
Scinaia, 509
Scytonema, 518
Scytosiphon, 505
Seirospora, 512
Sorapion, 503
Sorocarpus, 503
Spermatochnus, 504
Spermothamnion, 512
Sphacelaria, 506
Sphacella, 506
Sphaerococcus, 509
Sphaerotrichia, 504
Sphondylothamnion, 512
Spirulina, 517
Spongomorpha, 500
Spongonema, 503
Sporochnus, 505
Spyridia, 512
Stenogramme, 510
Stichococcus, 498
Stictyosiphon, 504
Stilophora, 504
Stilopsis, 504
Streblonema, 503
Strepsithalia, 504
Striaria, 504
Stypocaulon, 506
Sykidion, 498
Symphoricoccus, 504
Symphyocarpus, 504
Symploca, 517
Synechococcus, 515
Taonia, 506
Tellamia, 499
Thallochrysis, 502
Tilopteris, 505
Tolypothrix, 516
Trailliella, 511
Trebouxia, 498
Tribonema, 501
Ulonema, 504
Ulothrix, 498
Ulva, 498
Ulvella, 499
Urospora, 500
Vaucheria, 501
Waerniella, 503
Xenococcus, 516
Zanardinia, 505

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